DIVERSITY OF WORMWOOD (*ARTEMISIA ABSINTHIUM* L.) GROWING WILD IN POLAND IN RESPECT OF THE CONTENT AND COMPOSITION OF ESSENTIAL OIL AND PHENOLIC COMPOUNDS

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Introduction

Wormwood (*Artemisia absinthium* L., *Asteraceae)* is a perennial growing wild in ruderal communities, at roadsides and waste lands. Herb of this plant is used as a medicinal and flavouring raw material. It improves appetite and digestion, and reveals antihelminthic and antimicrobial activity. Bitter principles and essential oil are regarded to be the most important active compounds in wormwood herb [1-3]. Less is known about the content and biological activity of phenolic compounds in this raw material [4, 5]. In Poland wormwood herb is collected mainly from wild growing plants. Previous studies indicate that plants originating from different natural sites may differ considerably in respect of the composition of essential oil [6-11]. Special attention is called to the presence of thujone in wormwood essential oil, due to its neurotoxic activity [12]. Evaluation of intraspecific chemical diversity is important for the selection of populations that could be used as a source of raw materials for different applications (e.g. medicine, food industry, plant protection).

The aim of the study was to compare the content and composition of essential oil and phenolic compounds in herb obtained from eighteen populations of wormwood from central and north-eastern area of Poland.

Materials and methods

Wormwood herb (upper parts of shoots) was collected at the stage of plant blooming (mid August) from eighteen populations of wormwood occurring at natural sites in the central and north-eastern Poland (Table 1, Fig. 1-4), and dried naturally.

Content of essential oil in air-dry herb was determined by hydrodistillation method. The qualitative analysis of essential oil was carried out by gas chromatography (HP 6890, capillary column Carbowax 20 M 25 m, diameter 0.32 mm, carrier gas – helium). The separated constituents were identified by comparison of their retention times with the retention times of available standards. Sabinyl acetate, chrysanthenyl acetate and Z-(myroxide) were identified on the basis of GC-MS.

Methanolic extracts obtained by continuous exhaustive extraction of raw materials were used for qualitative and quantitative analysis of phenolic compounds by HPLC. The analysis was carried out using a Shimadzu chromatograph with SPD-M10A VP DAD detector and a Supelco LC RP 18 column (5 µm, 250 mm × 4.6 mm). The gradient of 10% acetonitrile in water and 55% acetonitrile in water at pH 3 was applied. Peaks were identified by comparison of retention time and spectral data with adequate parameters of standards. Quantification was based on the peak area. The following detection wave length was used: 206 nm (epigallocatechin gallate), 254 nm (diosmetin, orientin, hyperoside), 280 nm (p-coumaric acid), 330 nm (apigenin; caffeic, ferulic, chlorogenic and rosmarinic acids), and 370 nm (quercetin).

Table 1. Origin of the investigated populations

	Population/Location	Geographic coordinates	Land use
1	Czarny Las	51° 58.397′ N 21° 06.292′ E	waste land along the railroad
2	Tarnów	51° 47.427′ N 21° 26.480′ E	waste land abandoned farm
3	Podskwarne	52° 06.423′ N 21° 47.740′ E	pasture
4	Kołacz	52° 05.295′ N 21° 49.285′ E	roadside
5	Żebrak	52° 04.107′ N 22° 05.599′ E	roadside
6	Kaczory	52° 03.443′ N 22° 15.969′ E	waste land along the dirt-track
7	Szydłówka	52° 07.981′ N 22° 37.172′ E	waste land abandoned farm
8	Litewniki	52° 15.577′ N 22° 56.590′ E	waste land
9	Drohiczyn	52° 23.592′ N 22° 38.786′ E	pasture
10	Krzemień	52° 31.503′ N 22° 28.698′ E	meadow down by the forest
11	Sokoły Jeziorne	53° 37.893′ N 22° 16.862′ E	waste land abandoned farm
12	Gorczyce	53° 39.489′ N 22° 21.697′ E	pasture
13	Sokółki	53° 40.327′ N 22° 23.134′ E	knoll at the border between field and meadow
14	Rymki	53° 44.594′ N 22° 10.119′ E	forest nursery
15	Piasutno	53° 36.161′ N 21° 12.301′ E	waste land at the dirt-track
16	Jerutki	53° 35.402′ N 21° 08.756′ E	forest margin close to the road
17	Wały	53° 30.053′ N 21° 07.176′ E	roadside margin of the pasture
18	Wielbark	53° 23.814′ N 20° 57.502′ E	waste land next to the industrial area

Fig. 1. Location of natural sites of the investigated populations

Fig. 2. Population 1 (Czarny Las)

Fig. 3. Population 5 (Żebrak)

Fig. 4. Population 16 (Jerutki)

Results

The content of essential oil in the herb obtained from the investigated populations ranged from 0.40 to 0.88% (Fig. 5). According to Polish and European Pharmacopoeia the content of essential oil in this raw material shouldn't be lower than 0,20%. The populations differed in respect of the composition of essential oil (Table 2). These differences were not clearly related to the geographic origin of the populations. In six populations (no. 4, 6, 11, 13, 15, and 18) the dominant constituent of essential oil was sabinyl acetate, in three (3, 10 and 17) – chrysanthenyl acetate, in two (8 and 9) – sabinene, and in one (5) – β-thujone. Other populations were characterised by comparable content of two or three main compounds in essential oil, e.g. sabinene and β-myrcene, β-myrcene and cineol, chrysanthenyl acetate and β-myrcene, sabinyl acetate, chrysanthenyl acetate and β-thujone.

In the investigated raw materials eleven phenolic substances were identified: five phenolic acids (caffeic, p-coumaric, ferulic, chlorogenic, and rosmarinic one), five flavonoids (apigenin, diosmetin, orientin, quercetin, and hyperoside), and (-)-epigallocatechin gallate (Table 3). The presence of caffeic, ferulic and chlorogenic acid in wormwood herb has been previously reported [4]. In the raw materials obtained from all the investigated populations the dominant phenolic compound was ferulic acid but its content was diverse (396-1118 mg × 100 g^{-1}). Remarkable amount of chlorogenic and rosmarinic acids was also found (159-477 and 178-446 mg × 100 g^{-1}, respectively).

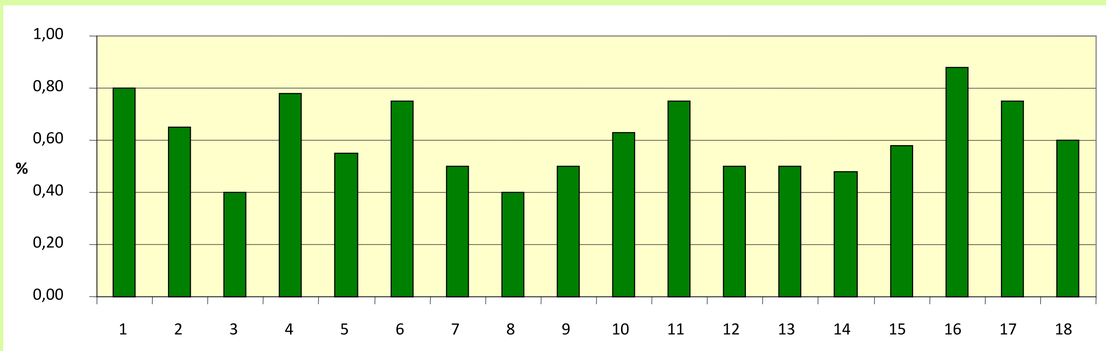

Fig 5. Content of essential oil in herb (%)

Table 2. Main constituents of essential oils (% in essential oil)

Compound	Population																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
α-pinene	2.55	2.89	4.33	2.26	2.08	2.27	2.36	1.63	0.82	4.66	0.50	2.54	1.44	2.48	1.93	2.72	1.58	1.33
sabinene	16.36	6.78	13.00	6.10	9.71	7.77	12.59	17.38	18.95	13.08	7.20	6.34	8.06	6.02	4.67	4.71	13.21	14.31
β-myrcene	13.86	20.30	14.91	4.86	1.74	9.06	13.05	8.72	8.37	14.71	8.07	16.33	5.64	4.65	4.14	4.62	4.10	9.60
cineol	5.12	10.78	5.80	3.27	10.97	7.29	3.28	4.94	13.09	10.98	3.90	14.87	5.90	11.37	7.91	8.95	2.23	6.76
p-cymene	1.61	0.54	2.33	0.50	1.44	0.74	1.38	2.46	3.40	1.33	1.07	1.21	1.09	1.06	0.18	1.21	1.68	1.29
β-thujone	0.06	0.10	nd	0.07	17.37	6.74	3.21	0.41	0.07	4.40	2.25	5.34	0.13	14.01	0.06	13.16	nd	0.40
(Z)-myroxide	8.35	nd	0.11	nd	0.80	nd	4.56	nd	0.15	nd	nd	nd	nd	nd	nd	0.20	nd	0.85
linalool	6.41	8.51	9.30	6.47	7.34	4.95	7.96	9.45	6.69	6.54	5.99	10.27	5.41	7.14	7.14	7.40	13.14	10.03
β-caryophyllene	1.07	0.56	1.01	0.74	0.75	0.76	0.74	0.68	0.91	0.67	0.78	1.00	0.70	1.28	0.96	0.88	1.44	0.54
terpinene-4-ol	2.20	1.03	2.22	1.41	2.16	1.46	2.62	7.83	3.32	2.12	1.84	1.52	1.92	1.95	1.90	1.22	4.25	3.00
sabinyl acetate	5.86	nd	0.22	39.74	7.55	24.13	5.30	1.42	14.84	0.18	44.12	7.98	44.10	15.77	30.36	17.63	11.97	23.00
α-terpineol	1.02	1.71	1.43	0.88	2.06	1.30	1.49	1.50	2.65	1.57	0.55	2.70	0.49	1.44	0.99	1.14	0.58	1.29
chrysanthenyl acetate	10.97	26.13	24.63	5.73	8.47	7.29	11.51	9.46	3.38	18.13	1.61	10.12	1.56	13.96	11.13	17.38	20.18	7.94
caryophyllene oxide	0.71	0.42	0.51	0.22	0.76	0.43	0.64	0.72	0.51	0.37	0.59	0.56	0.49	0.61	0.59	0.39	1.04	0.28
carvacrol	0.14	nd	nd	nd	0.71	1.81	1.03	1.64	0.14	0.10	nd	0.16	nd	nd	0.10	0.36	0.14	nd
α-bisabolol	1.07	1.10	1.04	nd	1.06	1.41	1.75	1.30	1.89	0.91	0.95	0.99	0.78	0.86	0.60	0.76	0.77	1.83
myristicin	0.20	0.23	0.86	1.01	0.36	0.79	0.79	1.06	0.21	0.72	0.13	0.34	0.81	0.37	0.33	0.44	0.46	0.47

nd – not detected; – clearly dominating constituent of essential oil; – constituents present in considerable amount

Table 3. Content of identified phenolic compounds in herb (mg × 100 g^{-1})

Phenolic compounds		Population																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
FLAVONOIDS	apigenin	1.4	1.3	3.7	1.2	0.9	0.9	1.1	1.8	0.9	0.9	0.8	0.6	3.1	0.8	1.1	2.6	0.6	3.1
	diosmetin	12.3	15.2	13.0	16.9	15.3	17.2	14.1	14.1	12.5	12.6	13.9	30.1	14.8	14.7	14.7	21.0	14.3	12.6
	orientin	33.1	14.7	22.0	28.7	21.9	30.1	12.9	17.8	19.5	28.7	18.5	16.0	14.8	7.3	6.7	18.9	16.2	27.6
	quercetin	0.5	0.2	0.7	0.2	0.4	0.6	0.1	0.5	0.3	0.1	0.2	0.9	0.7	0.2	0.1	0.7	0.6	0.7
	hyperoside	40.1	31.6	43.9	18.2	37.0	41.8	29.4	38.5	38.7	33.7	37.0	36.0	28.4	43.7	34.0	34.4	37.8	39.8
PHENOLIC ACIDS	caffeic acid	10.1	3.1	3.0	3.7	3.5	4.5	5.5	2.1	5.6	3.5	6.4	5.1	6.3	4.6	3.4	2.8	9.7	5.3
	p-coumaric acid	16.6	16.4	16.6	16.7	16.1	16.4	19.3	16.2	16.0	16.6	21.8	17.1	16.2	22.8	20.3	16.0	22.5	16.6
	ferulic acid	459.8	396.2	848.2	777.6	650.2	1027.8	570.5	901.7	705.3	742.2	914.2	1118.5	895.4	1109.1	839.2	853.4	981.7	1013.9
	chlorogenic acid	159.3	241.5	332.1	227.5	247.5	477.1	170.4	226.0	286.6	297.7	360.6	412.5	174.6	436.7	263.0	328.3	445.1	467.9
	rosmarinic acid	195.5	177.8	333.5	295.5	222.8	369.5	257.7	292.6	274.2	234.8	345.8	445.8	312.6	391.0	307.5	256.7	445.2	337.1
(-)-epigallocatechin gallate		42.3	41.7	44.5	44.5	48.6	49.0	51.3	42.1	48.1	49.1	47.9	42.1	43.5	51.1	51.1	44.6	47.8	42.8

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