ANALYSIS OF LIPOPHILIC FRACTION FROM LEAVES, INFLORESCENCES AND RHIZOMES OF *SILPHIUM PERFOLIATUM* L.

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ABSTRACT

This paper presents a qualitative and quantitative chromatographic analysis (GC/MS) of the lipophilic fraction extracted from leaves, inflorescences and rhizomes of *Silphium perfoliatum* L. collected in 1998-2000.

It was shown that the extracts of leaves and inflorescences are similar in composition, containing among their main constituents: α-amyrine (up to 13.9% of total components in leaves, up to 11.3% in inflorescences), heptacosane (up to 7.1% in leaves, up to 7.6% in inflorescences), stigmasterol (up to 8.1% in leaves, up to 2.2% in inflorescences), γ-sitosterol (up to 6.9% in leaves, up to 2.2% in inflorescences), β-amyrine (up to 4.1% in leaves, up to 5.8% in inflorescences), β-caryophyllene (up to 2.6% in leaves, up to 1.5% in inflorescences), caryophyllene oxide (up to 4.4% in leaves, up to 2.9% in inflorescences), germacrene D (up to 13.8% in leaves, up to 9.7% in inflorescences) and α-pinene (up to 3.3% in leaves, up to 2.4% in inflorescences).

The chemical composition of lipophilic extract of rhizomes significantly differs from that of leaves and inflorescences. The following compounds can be counted among the dominant ones occurring in rhizome extracts: diterpene of labdane type – 16-acetoxycarterochaetol (up to 45%) and sesquiterpenes – 7-β-H-silphiperfol-5-ene (up to 9.1%), 7-α-H-silphiperfol-5-ene (up to 10.7%), δ-elemene (up to 7.6%), isocomene (up to 5.6%), germacrene D (up to 5.2%) and modhephene (up to 5.1%).

KEY WORDS: *Silphium* L., *Silphium perfoliatum* L., Asteraceae, GC/MS, lipophilic fraction, sesquiterpenes.

INTRODUCTION

The paper presented here is a fragment of a multidisciplinary study cycle carried out by the author on a North-American species of *Silphium* L. genus (Asteraceae) (Weryszko-Chmielewska et al. 1999a, b; Wolski et al. 1999, 2000; Kowalski 2001, 2002, 2003; Kowalski, Wolski 2001, 2003a, b, in press – 2004 online; Kowalski, Wierciński 2003; Kowalski et al. 2005 – in press).

The aim of the present paper was to analyze the lipophilic fraction of leaves, inflorescences and rhizomes of the cup plant (*Silphium perfoliatum* L.) cultivated in the Lublin region as a potential herbal or fodder raw material. The research carried out is of an experimental nature and enables the future categorisation of *Silphium* varieties by their chemotaxonomy.

MATERIALS AND METHODS

Plant material

The study was conducted making use of leaves, inflorescences and rhizomes of *Silphium perfoliatum* L., originating from ten-year old plants (1998), grown in the Department of Botany, University of Agriculture and from two-year old plants (1999-2000), grown in the Department of Vegetable and Medicinal Plants, University of Agriculture in Lublin. The leaves and inflorescences were collected in July, and rhizomes in October. They were dried in shade and air and then ground.

Extraction

Extracts for study were obtained by extraction of 40 g powdered raw material using petroleum ether (1:10) in Soxhlet's apparatus (12 hrs, 60°C). Extracts were filtered

and subsequently subjected to GC/MS analysis. In addition, the contents of the dry ether extract were evaluated by mass after prior solvent evaporation at a temperature of 60°C.

GC/MS

The GC/MS instrument ITS-40 (GC/ITMS – Finnigan MAT, USA) was used, with 30 m × 0.25 mm i.d., DB-5 column (J&W, USA), film thickness 0.25 mm, carrier gas He, injector temperature -280°C. A temperature gradient was applied (35°C for 2 minutes, then increased by 4°C/min to 280°C). The qualitative analysis was carried out on the basis of MS spectra which were compared with the spectra of the NIST library (1992) and the LIBR (TR) terpenes library provided by Finnigan MAT (1990), and data available in literature (Bohlmann, Jakupovic 1979, 1980; Bohlmann et al. 1977, 1979a, b; Careri et al. 2001; Killops, Frewin 1994; Kowalski, Wolski in press – 2004 online; Joulain, König 1998; Zalkow et al. 1977, 1978; SCRI 2003). Identity of the compounds was confirmed by their retention indices taken from literature and own data (Kowalski 2001; Menut et al. 1997; Weyerstahl et al. 1997; Joulain, König 1998; Kowalski, Wolski in press – 2004 online; Palá-Paúl et al. 2002; Tellez et al. 2002).

The retention index defines the analyte peak location on the chromatogram with regard to the n-alkanes:

$$I_X = (t_{Rx} - t_{Rn}) \div (t_{Rn+1} - t_{Rn}) \times 100 + 100n$$

where: t_{Rx} – the analyte retention time, t_{Rn} – the retention time of the n-alkane with n-carbon atoms, t_{Rn+1} – the retention time of alkane with n+1 carbon atoms and $t_{Rn} \leq t_{Rx} \leq t_{Rn+1}$.

The quantitative composition of extracts was determined assuming the total of the particular extracts to constitute 100%.

RESULTS AND DISCUSSION

Petroleum ether extracts from leaves, inflorescences and rhizomes differed organoleptically one from another. Extracts produced from rhizomes were the most viscous and were characterised by a transparent amber colour and an odour recalling coniferous tree resin. Extracts prepared from leaves and inflorescences had a fatty-resin consistence and undetermined odour, as well as a green colour in the case of leaves and yellowish-brown colour in the case of inflorescences. Moreover, the percentage of the extracted dry matter recalculated onto studied organic dry matter was at the following levels: 1.70% for leaves, 4.53% for inflorescences and 2.52% for rhizomes (Table 1).

GC/MS chromatograms of the components of the extracts from leaves, inflorescences and rhizomes of cup plants are presented in Figure 1. Table 2 lists the percentage of particular components of studied materials.

TABLE 1. Percentage of the *Silphium* petroleum ether extract dry matter recalculated onto studied organic dry matter.

Organs	Content of *Silphium* petroleum ether extract (%DM)			
	1998	1999	2000	Mean
Leaves	1.62	1.55	1.93	1.70
Inflorescences	4.65	4.27	4.67	4.53
Rhizomes	2.62	2.37	2.52	2.52

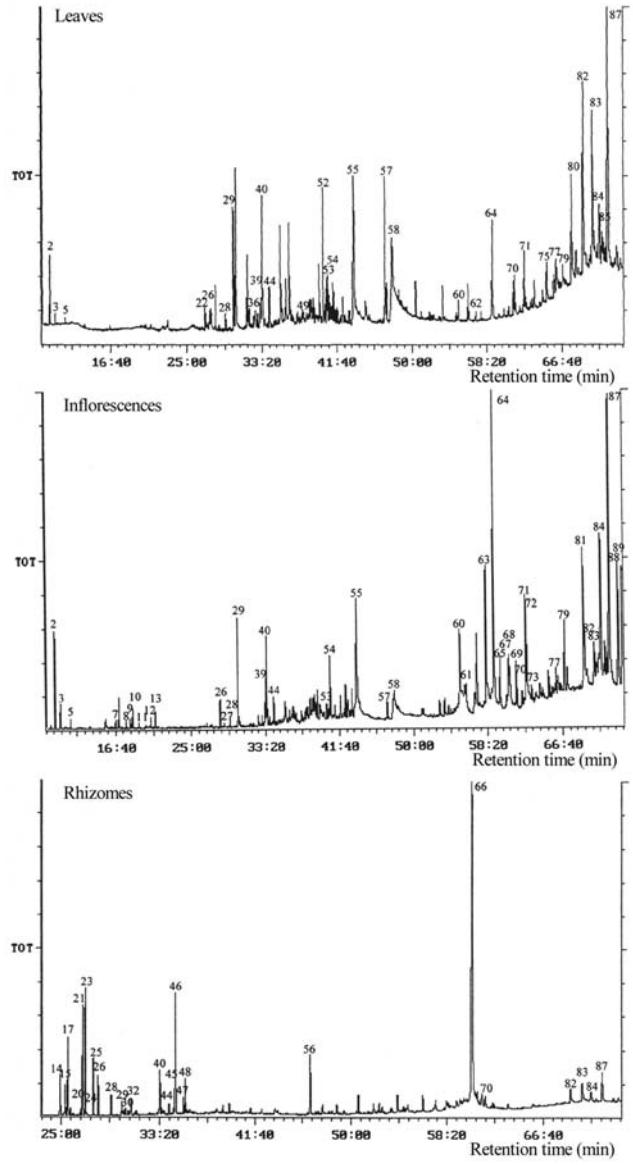

Fig. 1. GC/MS chromatograms of ingredients of lipophilic extracts made from leaves, inflorescences and rhizomes of cup-plant *Silphium perfoliatum* L. (1999) – individual compounds were marked in accordance to Table 2.

GC/MS analysis of the extracts isolated from leaves and inflorescences showed that they were of similar composition. The following compounds were identified in both leaf and inflorescence extracts: α-amyrine (triterpene alcohol), heptacosane (alkane), stigmasterol and γ-sitosterol (phytosterols), β-amyrine (triterpene alcohol). The occurrence of α-amyrine – characteristic of plants producing resins and alkanes – in components of the cuticle covering the skin of the aboveground parts of plants, is noteworthy. Moreover, several chemical compounds that were earlier found in essential oils isolated from these raw materials (Wolski et al. 2000; Kowalski, Wolski in press – 2004 online), among others: β-caryophyllene caryophyllene oxide, germacrene D and α-pinene, were present in the studied extracts.

The chemical composition of the petroleum ether extracts from rhizomes significantly differs from that of leaves and inflorescences. Labdane type diterpene (16-aceto-

TABLE 2. The percentage of main components of lipophilic extracts from leaves, inflorescences and rhizomes of the cup-plant (*Silphium perfoliatum* L.) using petroleum ether.

No.	Compound	Retention index	Percentage share of the ingredients of lipophilic extracts (%)									Reference
			Leaves			Inflorescences			Rhizomes			
			1998	1999	2000	1998	1999	2000	1998	1999	2000	
1	tricyclene	919.2	-	-	0.1	-	tr.	0.1	-	-	-	N
2	α-pinene	931.2	0.2	1.5	3.3	0.3	1.2	2.4	-	-	-	N
3	camphene	945.8	0.2	0.2	0.5	-	0.3	0.4	-	-	-	N
4	sabinene	971.5	-	-	tr.	-	-	tr.	-	-	-	N
5	β-pinene	974.7	0.2	0.1	-	0.2	0.1	0.2	-	-	-	N
6	limonene	1029.1	tr.	-	tr.	tr.	tr.	tr.	-	-	-	N
7	α-pinene oxide	1100.4	0.2	-	0.2	0.2	0.1	0.2	-	-	-	N
8	α-campholenal	1130.4	-	-	0.1	-	0.1	-	-	-	-	N
9	trans-pinocarveol	1142.8	-	-	0.1	0.1	0.1	-	-	-	-	N
10	trans-verbenol	1149.7	-	-	0.4	0.2	0.3	0.3	-	-	-	N
11	pinocarvone	1168.1	-	-	0.1	0.1	0.1	tr.	-	-	-	N
12	myrtenal + myrtenol	1202.1	-	-	0.2	0.1	0.2	0.1	-	-	-	N
13	verbenone	1216.7	0.2	-	0.4	0.2	0.3	0.3	-	-	-	N
14	7-α-H-silphiperfol-5-ene	1327.8	0.2	-	-	0.2	-	-	10.7	1.9	5.4	2, 7
15	δ-elemene	1340.6	tr.	-	-	0.1	-	-	0.2	0.1	7.6	7
16	7-β-H-silphiperfol-5-ene	1343.6	0.2	-	-	tr.	-	-	5.7	-	9.1	2, 7
17	n.i.	1347.3	-	-	-	-	-	-	2.6	4.0	-	
18	silphiperfola-5,7 (14)-diene	1361.3	-	-	-	-	-	-	0.9	0.1	-	7
19	α-copaene	1379.6	0.3	-	0.2	0.3	tr.	0.2	0.3	-	-	7
20	silphiperfol-6-ene	1379.8	tr.	-	-	-	-	-	0.7	0.3	0.7	2, 7, 11
21	modhephene	1384.7	-	-	-	-	-	-	2.1	5.1	1.2	3, 17
22	β-bourbonene	1389.2	1.2	0.5	tr.	tr.	-	-	-	-	-	7
23	isocomene	1391.9	-	-	-	-	-	-	2.4	5.6	0.7	4, 11
24	β-elemene	1395.8	0.3	-	-	0.2	-	-	-	0.1	0.1	16
25	β-isocomene	1412.3	-	-	-	-	-	-	0.5	2.6	0.3	7
26	β-caryophyllene	1424.8	2.6	1.2	1.2	1.5	0.5	1.0	0.6	1.8	2.0	7, 15, 11
27	trans-α-bergamotene	1439.2	0.4	tr.	0.1	0.1	0.1	0.1	-	-	-	7
28	α-humulene	1459.8	0.7	0.5	0.3	0.4	0.2	0.2	1.2	0.9	2.4	11, 15
29	germacrene D	1487.5	13.5	3.2	4.3	9.1	1.7	3.6	0.5	0.6	5.2	7, 15
30	δ-selinene	1497.4	-	-	-	-	-	-	0.7	0.4	-	7
31	germacrene B	1499.2	0.2	-	tr.	0.1	-	-	-	-	-	7
32	β-bisabolene	1512.9	-	-	-	-	-	-	1.4	0.8	0.7	7
33	γ-cadinene	1516.5	tr.	-	tr.	-	-	-	tr.	tr.	-	7
34	δ-cadinene	1529.9	0.3	-	tr.	0.2	tr.	-	0.3	tr.	-	7
35	γ-elemene	1566.2	-	-	-	-	-	-	tr.	tr.	-	7
36	(E)-nerolidol	1568.1	0.8	0.1	tr.	0.1	tr.	tr.	-	-	-	N
37	n.i.	1576.4	0.4	0.4	0.2	0.2	0.2	0.2	-	tr.	-	10
38	4-β-hydroksygermacra-1 (10),5-diene	1584.6	-	-	tr.	-	tr.	tr.	-	tr.	-	10
39	spathulenol	1587.6	1.5	1.1	0.5	0.7	0.4	0.5	tr.	-	-	11
40	caryophyllene oxide	1593.1	1.8	4.4	3.1	1.2	2.9	2.8	3.0	1.8	0.3	N
41	n.i.	1596.4	1.0	0.4	0.4	0.5	0.3	0.4	0.2	-	-	10
42	salvial-4 (14)-en-1-one	1604.1	0.1	0.1	0.2	0.1	0.1	-	0.2	0.4	-	10
43	globulol	1610.7	-	-	-	-	-	0.2	-	-	-	12
44	humulene epoxide II + β-oplopenone	1620.3	0.9	1.8	1.0	0.6	0.8	0.9	2.9	0.6	-	7
45	2,3,5,9-tetramethyl-tricyclo-[6.3.0.0E1,5]-undek-2-en-4-one	1635.1	-	-	-	-	-	-	5.0	0.2	0.2	N
46	n.i.[a]	1640.0	0.3	tr.	0.2	0.5	-	0.1	4.0	5.9	2.6	10
47	aplotaxene	1664.1	-	-	-	-	-	-	0.4	0.7	0.6	9
48	n.i.	1669.6	-	-	-	-	-	-	0.5	1.7	1.0	10

TABLE 2. Cont.

No.	Compound	Retention index	Percentage share of the ingredients of lipophilic extracts (%)									Reference
			Leaves			Inflorescences			Rhizomes			
			1998	1999	2000	1998	1999	2000	1998	1999	2000	
49	oplopanone	1752.1	0.3	0.5	-	0.2	0.1	-	-	-	-	9
50	oplopenone	1754.0	tr.	-	tr.	-	-	-	-	-	-	9
51	n.i.	1780.8	0.4	0.6	0.5	0.3	0.4	0.7	-	-	-	
52	n.i.	1838.5	1.1	3.5	0.2	0.7	0.3	0.2	-	-	-	
53	6,10,14-trimethyl-2-pentadecanone	1847.1	0.9	0.6	0.3	0.2	0.2	0.4	-	-	-	N
54	n.i.	1853.5	1.7	1.3	0.8	1.1	1.1	0.9	-	-	-	10
55	hexadecanoic acid	1970.1	9.2	2.0	4.0	7.6	3.7	4.2	-	-	-	N
56	n.i.	2093.6	-	-	-	-	-	-	5.7	3.5	-	
57	phytol	2115.9	2.9	5.1	0.5	0.5	0.3	0.6	-	-	-	14
58	(Z,Z,Z)-9,12,15-octadecatrienoic acid methyl ester	2155.8	11.2	5.8	0.6	4.3	0.8	1.8	-	-	-	N
59	n.i.	2188.0	-	-	-	-	-	-	-	-	1.6	N
60	pentacosane	2495.5	0.6	0.5	2.3	1.3	2.0	2.5	-	-	-	N
61	docosanoic acid methyl ester	2530.9	-	-	1.5	0.5	0.8	1.5	-	-	-	N
62	hexacosane	2595.3	0.7	0.2	-	-	-	-	-	-	-	N
63	n.i.	2653.0	tr.	-	5.7	5.0	3.4	5.9	-	-	-	N
64	heptacosane	2695.6	1.9	6.3	7.1	7.3	6.9	7.6	-	-	-	N
65	tetracosanic acid methyl ester	2737.0	0.6	-	2.6	2.2	0.9	2.6	-	-	-	N
66	16-acetoxycarterochaetol	2786.6	-	-	-	-	-	-	32.1	31.7	45.0	1
67	n.i.	2789.0	-	-	1.1	1.0	1.4	1.0	-	-	-	
68	octacosane	2794.5	0.3	tr.	0.7	0.7	0.8	0.8	-	-	-	N
69	n.i.	2795.0	-	-	0.9	1.0	1.3	0.9	-	-	-	
70	squalene	2838.0	1.0	1.2	0.9	0.9	0.8	0.8	0.3	0.4	-	13
71	nonacosane	2900.0	1.1	1.5	2.6	4.2	2.1	2.6	-	-	-	N
72	n.i.	2909.0	0.4	-	2.1	0.4	1.5	2.0	-	-	-	
73	hexacosanoic acid methyl ester	2940.0	0.2	tr.	0.7	0.8	0.3	0.7	-	-	-	N
74	triacontane	3000.0	0.2	tr.	-	0.9	tr.	-	0.5	-	-	N
75	n.i.	3052.0	0.6	1.5	0.4	tr.	0.6	-	-	-	-	
76	β-tocopherol	3076.1	-	-	-	0.1	0.1	-	-	-	-	N
77	hentriacontane	3100.0	0.3	0.6	0.8	0.4	0.5	0.7	-	-	-	N
78	octadecanoic acid methyl ester	n.d.	-	-	0.3	-	-	0.3	-	-	-	N
79	vitamin E	n.d.	0.3	0.4	1.5	0.9	1.4	1.4	-	-	-	N
80	n.i.	n.d.	1.0	5.3	-	-	-	-	-	-	-	
81	n.i.	n.d.	-	-	-	0.4	3.3	4.2	-	-	-	
82	stigmasterol	n.d.	7.5	8.1	2.4	1.6	1.9	2.2	1.0	1.0	0.6	N, 5
83	γ-sitosterol	n.d.	5.1	6.9	1.7	1.1	1.2	0.9	1.3	1.5	0.4	N, 5
84	β-amyrine	n.d.	2.0	2.9	4.1	3.9	5.8	3.8	0.5	0.9	0.4	N, 6, 8
85	n.i.	n.d.	-	1.0	-	-	0.9	-	-	0.1	-	
86	n.i.	n.d.	-	0.3	-	-	1.2	-	-	-	-	
87	α-amyrine	n.d.	11.8	13.9	6.9	10.1	11.3	6.3	2.0	2.5	2.6	N, 6, 8
88	n.i.	n.d.	0.3	0.4	1.9	1.9	3.8	1.7	0.3	0.3	-	
89	n.i.	n.d.	tr.	tr.	-	tr.	3.9	-	tr.	0.3	-	
90	n.i.	n.d.	-	1.3	-	-	1.8	-	-	-	-	
91	n.i.	n.d.	-	tr.	-	-	1.2	-	-	-	-	
92	n.i.	n.d.	-	0.6	-	-	4.5	-	-	1.0	-	

„n.i." – not identified; „tr." – trace (<0.1%); „n.d." – not determined; [a] – spectrum similar to that of spathulenol; N – NIST library and terpene library LIBR (Finnigan MAT); 1) Bohlmann, Jakupovic 1979; 2) Bohlmann, Jakupovic 1980; 3) Bohlmann et al. 1979a; 4) Bohlmann et al. 1977; 5) Careri et al. 2001; 6) Chosson et al. 2003; 7) Joulain, König 1998; 8) Killops, Frewin 1994; 9) Kowalski 2001; 10) Kowalski, Wolski in press – 2004 online; 11) Menut et al. 1997; 12) Palá-Paúl et al. 2002; 13) SCRI 2003; 14) Tellez et al. 2002; 15) Weyerstahl et al. 1997; 16) Zalkow et al. 1977; 17) Zalkow et al. 1978.

xycarterochaetol) and sesquiterpene compounds (7-β-H--silphiperfol-5-ene, δ-elemene, isocomene, silphiperfol-6--ene, germacrene D, modhephene) dominated in rhizome extracts. Moreover, the presence of compounds occurring in oils isolated from the raw material was found. Previously, Bohlmann and Jakupovic (1979, 1980) had found that extracts made from *Silphium perfoliatum* L. rhizomes contained sesquiterpene hydrocarbons, including: aplotaxene, germacrene, eudesmane and guayane derivatives, modhephene, silphinene, silphiperfol-6-ene, 7-α-H-silphiperfol-5--ene, 7-β-H-silphiperfol-5-ene, isocomene. Labdane type diterpenes and carterochaetol derivatives were a remarkable group of isoprenoid compounds in rhizomes. Our results are similar to the above data.

It is noteworthy that the percentage of particular components of the extracts obtained from the above and below-ground parts of the cup plant varied depending on the plant's age and the study year, which can be accounted for by fluctuations in physiological and environmental factors. As regards the quality, the extracts studied showed no differences which were dependent on the year of experiment. Comparing the volatility of compounds isolated from leaves, inflorescences and rhizomes of the cup plant, it is demonstrated that volatile (low-molecular) compounds were present only in the aboveground organs (e.g. tricyclene, α-pinene, camphene, β-pinene, p-cymene, limonene, α-campholenal, cis-verbenol, trans-pinocarveol, trans-verbenol, pinocarvone, myrtenal, myrtenol and verbenone).

The presented paper describes for the first time the composition of total lipophilic fraction (petroleum ether) of leaves, inflorescences and rhizomes of *Silphium perfoliatum* L. obtained from two- and ten-year old plants in cultivation experiments carried out in the period 1998-2000. The results may be useful for the evaluation of plants in relation to their biological activity, which will be discussed in the next paper.

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