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Studies of Selected Plant Raw Materials as Alternative Sources of Triterpenes of Oleanolic and Ursolic Acid Types

RADOSŁAW KOWALSKI*

Department of Analysis and Evaluation of Food Quality, Central Apparatus Laboratory, University of Agriculture, 13 Akademicka Street, 20-950 Lublin, Poland

The qualitative and quantitative evaluation of triterpene aglycones of saponin fractions isolated from vegetative and generative organs of three *Silphium* species, *Silphium perfoliatum*, *Silphium trifoliatum*, and *Silphium integrifolium*, as compared to materials used in the herbal industry such as *Panax quinquefolium* root and *Calendula officinalis* flower, was performed. The analyses revealed that triterpene aglycones of saponins isolated from tested *Silphium* and *Calendula* species were oleanolic acid and ursolic acid. It was found that *Panax* roots contained only the aglycone of oleanolic acid within the triterpene saponin group. The leaves of *Silphium* harvested in May were characterized by the highest content of oleanolic acid—They contained 17.03 mg/g dry weight of the triterpenic acid, on average. The seasons before flowering and at the beginning of that stage appeared to be the most efficient periods for leaf collection in reference to triterpene aglycone contents in plant yield. Moreover, it was found that inflorescences of *S. trifoliatum* and *S. integrifolium* contained oleanolic acid in amounts of 22.05 and 17.95 mg/g dry weight respectively, whereas *Calendula* flowers contained 20.53 mg/g dry weight. The oleanolic acid content in *Panax* roots was 3.15 mg/g dry weight. Ursolic acid most abundantly occurred in *S. integrifolium* and *S. trifoliatum* at concentrations of about 14.98 mg/g dry weight in leaves harvested before flowering (June) and to 15.50 mg/g dry weight in leaves collected during flowering.

KEYWORDS: Oleanolic acid; ursolic acid; triterpenes; saponins; *Silphium perfoliatum*; *Silphium trifoliatum*; *Silphium integrifolium*; *Calendula officinalis*; *Panax quinquefolium*

INTRODUCTION

Oleanolic (3β-hydroxy-olea-12-en-28-oic acid) and ursolic (3β-hydroxy-urs-12-en-28-oic acid) acids are isomeric triterpene compounds occurring in the plant kingdom as free acids or aglycones of triterpene saponins (*1*). The literature states that oleanolic and ursolic acids show antibacterial (*2*, *3*), antifungal (*4*, *5*), insecticidal (*6*), anti-HIV (*7*, *8*), complement inhibitory (*9*), diuretic (*10*), antidiabetogenic (*11*), and gastrointestinal transit modulating activities (*12*). Moreover, oleanolic and ursolic acids have protective action to liver (*1*), antiinflammatory effects (*13*), antitumor activity (*1*, *14*−*16*), and immunomodulatory activity (*17*).

Up-to-date reports indicate that, besides commonly known plants, North American perennials of the *Silphium* L. genus may be an interesting source of oleanolic acid glycosides (*18*, *19*). It is worth mentioning that North American Indian tribes applied various organs of *Silphium perfoliatum* L. for medical purposes (*20*). The root of *S. perfoliatum* has tonic, diaphoretic, and alterative proprieties. It was found useful in liver and spleen maladies and also in fevers, internal bruises, debility, and ulcers. American Indians from the Fox tribe recommended the use of *Silphium integrifolium* rhizomes to treat kidney diseases and as an analgesic agent and used the brew from leaves in urinary bladder disturbances (*21*). Hitherto studies performed on the biological activity of ethanol extracts from *S. perfoliatum* showed their regenerative action during postscald wound healing in rats (*22*). An anticholesterol action of saponins isolated from *S. perfoliatum* leaves (so-called "silphiosides") was found as well. The cholesterol concentration in rat's blood decreased by 12 and 19% depending on the dose and the time course of the experiment (*23*). Moreover, Davidjanc et al. (*24*) found that saponins from *S. perfoliatum* leaves inhibited the development of phytopathogenic fungi *Drechslera graminea* (Rabh) Ito, *Rhizopus nodosus* Namysl, and *Rhizopus nigricans* Ehr.

The lack of detailed and systematic research on the content of valuable secondary triterpene metabolites of *Silphium* forces us to undertake studies within the subject. Therefore, the aim of present paper is the qualitative and quantitative evaluation of triterpene aglycones of saponin fractions isolated from vegetative and generative organs of three *Silphium* species, *S. perfoliatum*, *Silphium trifoliatum*, and *S. integrifolium*, as compared to materials used in the herbal industry such as American ginseng root (*Panax quinquefolium*) and pot marigold flower (*Calendula officinalis*) in a view of the potential

* To whom correspondence should be addressed. Tel: +48 81 445 66 57. Fax: +48 81 533 35 49. E-mail: radoslaw.kowalski@ar.lublin.pl.

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Table 1. Dates of Plant Material Harvest for Phytochemical Studies

plant material	harvest date	plant development stage
Silphium leaves	May 15, 2003	intensive growth
	June 15, 2003	flower buds
	July 15, 2003	beginning of flowering
Silphium inflorescences	July 15, 2003	beginning of flowering
Silphium seeds	Oct 4, 2003	fructification
Silphium rhizomes	Oct 4, 2003	fructification
Silphium roots	Oct 4, 2003	fructification
Calendula flowers	July 15, 2003	flowering
Panax roots	Oct 4, 2003	flowering and fructification

application of *Silphium* as a source of materials for the pharmaceutical industry.

MATERIALS AND METHODS

Plant Materials. The leaves, inflorescences, seeds, rhizomes, and roots of *S. perfoliatum*, *S. trifoliatum*, and *S. integrifolium* originating from a 3 year old experimental cultivation (2003) were proportionate by the Department of Analysis and Evaluation of Food Quality University of Agriculture (Lublin, Poland) in Kazimierzówka near Lublin (51°14′N, 22°34′E; altitude, 200 m). The plants were grown on a lessive soil developed from loess forms on lime marl containing 1.6% of organic matter; the surplus of the continent is influenced by the great amplitudes of annual temperatures as well as long summers and long cool winters in Lublin Upland and mineral fertilization at rates of N, 100; P, 80; and K, 100 kg/ha.

Anatomical and morphological traits of the species were described in the earlier papers (*25−28*). Moreover, the roots of 4 year old American ginseng (*P. quinquefolium* L.) and the flowers of pot marigolds (*C. officinalis* L.) originating from cultivation by the Department of Industrial and Medical Plants, University of Agriculture (Lublin, Poland) and herbal producer Herbost in Kębłow near Lublin served as reference plants. Voucher specimens of *P. quinquefolium* and *C. officinalis* were deposited at the Department of Industrial and Medical Plants, University of Agriculture. The harvest dates of studied organs are presented in **Table 1**. Fresh material was frozen and then lyophilized (Labconco lyophilizer) with subsequent grinding.

Extraction. The extraction of materials was made with modification of an earlier described procedure (*19*). Aliquots of 2.00 g of powdered materials (in four replications) were defatted using hexane in Soxhlet's apparatus for 4 h. Then, after it was dried, the material was extracted with 80% hot methanol (50 mL) (3 × 30 min) and the alcoholic extracts were collected. Combined methanol extracts were concentrated in a rotational evaporator at 60 °C. Amounts of 4 mL of deionized water were added to dense remains and thoroughly stirred, and then, 2 × 1.8 mL of solution was taken (extracts A and B) that was introduced onto columns Extrelut NT3 (Merck). After 10 min, the columns were washed with 15 mL of butanol saturated with water. Achieved filtrates A and B were concentrated in an evaporator at 70 °C. Dried extracts were dissolved in methanol and quantitatively transferred into glass vials (10 mL). The vials containing methanol extracts A and B were placed in a water bath (70 °C) to remove alcohol. Aliquots of 5 mL of methanol [high-performance liquid chromatography (HPLC) grade, POCh] were added into the vials with A extracts, and contents were thoroughly stirred till dissolved. The obtained extract was 10 times diluted with methanol (HPLC grade) and transferred into the vessels from which samples were taken to HPLC-photodiode array/electrospray ionization/mass spectrometry (PDA/ESI/MS) screening analysis.

Hydrolysis of Saponins and Silylation of Aglycones for Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. Volumes of 5 mL of methanol were added into the vials with extract B of saponins, and the contents were thoroughly stirred till dissolved. Aliquots of 50 μL were taken from every vial and transferred into the vessels hermetically closed with a PTFE seal (20 mL), and then, 4 mL of methanol solution of hydrochloric acid (HCl:MeOH, 1:6) was added with subsequent hydrolysis by heating at 100 °C for 4 h in a thermostat. Hydrolyzates were dried, and 3 mL of diethyl ether was added, shaken, and quantitatively transferred into the tap funnels (25 mL) by adding 20 μL of methanol solution of cholesterol (Sigma)—internal standard (2 mg/mL) as well as 5 mL of deionized water. The mixture was shaken and remained for some time, and after phase separation, the upper ether layer was collected. Another 3 mL of diethyl ether was added to the remaining water fraction, and the separation was twice repeated. The combined ether extracts were transferred into the tap funnels (25 mL) and washed with proportional amounts of deionized water till the neutral reaction of water fraction, which was checked by means of universal indicator paper. Washed ether fractions were quantitatively transferred into the vessels sealed with PTFE plugs, and then, the solvent was evaporated in a water bath (80 °C) in a fume cupboard. Aliquots of 200 μL of silanes mixture, N,O-bis(trimethylsilyl)-tri-fluoroacetamide (BSTFA, Merck), trimrthylchlorosilane (TMSCl, Merck), and trimethylsilylimidasol (TMSI, Merck) (1.5:1:1.5 v/v/v), were added to the dried remains and then heated in a thermostat at 70 °C for 20 min (*29*). The derivatized sample, after dilution to 1 mL using hexane, was directly subjected to GC-MS analysis.

Identification, Standard Preparation, and Quantitative Analysis. Screening of extract components from ginseng, silphium, and marigold was performed by comparison of their retention times and MS spectra for HPLC separations of studied extracts and standard solutions of glucuronide F (CLA), hederacoside C (Roth), and ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2, and Rd (Roth). Moreover, qualitative interpretation of isolated components was made by comparison of achieved mass spectra for particular separated substances with spectra and molecular weights for earlier described saponins from *Calendula* and *Panax* species (*30−33*). The quantitative composition of raw saponin extracts was determined by assuming the total of all of the particular components to be 100%.

Qualitative GC-MS analysis of silylo-derivative aglycones was carried out by comparison of retention times and MS spectra for GC separations of studied extracts and standard solutions of oleanolic acid (Sigma), ursolic acid (Sigma), and hederagenine (Roth) silylo-derivatives. The quantitative GC-MS analyses were performed on a base of calibration curves for mixtures of oleanolic acid, ursolic acid, hederagenine, and cholesterol silylo-derivatives (0.04−0.40 mg).

The procedure of saponin isolation, their hydrolysis, and aglycone silylation was controlled by performing the recovery in reference to standard saponins (0.25 mg) of glucuronide F (CLA), hederacoside C (Roth), and ginsenoside Rb_2 (Roth), which were subjected to described preparing stages taking into account the quantitative losses in final calculations. Moreover, oleanolic and ursolic acids as well as hederagenine were treated with a hydrolyzing mixture under the above-described conditions to exclude the effects of hydrolysis of artifacts formation at amounts of analytical importance.

HPLC-PDA/ESI/MS. An LC system consisting of a Finnigan Surveyour pump equipped with a gradient controller, an automatic sample injector, and a PDA detector was used. The separation was performed on a 250 mm × 4 mm i.d., 5 μm, Eurospher 100 C_{18} column (Knauer, Germany). A mobile phase consisted of 0.5% acetic acid in water (B), and 0.05% acetic acid in acetonitrile (A) was used for the separation. The flow rate was kept constant at 0.6 mL/min for a total run time of 110 min. The system was run with the following gradient program: 0.00−15 min, isocratic 20% A; 45 min, 46% A; 50 min, 55% A; 50−90 min, isocratic 55% A; 95 min, 90% A; 95−100 min, isocratic 90% A; 105 min, 20% A; and 105−110 min, isocratic. The sample injection volume was 10 μL.

A Thermo Finnigan LCQ Adventage Max ITMS with an electrospray ion source was coupled to the HPLC system described above. The samples were introduced on column via an automatic sampler injector or direct injection by a syringe pump at a flow rate of 5 μL/min. The spray voltage was set to 4.2 kV, and the capillary offset voltage was set to −60 V. All spectra were acquired at a capillary temperature of 220 °C. The calibration of the mass range (400−2000 Da) was performed in negative ion mode. Nitrogen was used as the sheath gas, and the flow rate was 0.9 L/min. The maximum ion injection time was set to 200 ms.

GC-MS. ITMS Varian 4000 GC-MS/MS (Varian, United States) equipped with a CP-8410 autoinjector and a 30 m × 0.25 mm VF-5ms column (Varian); film thickness, 0.25 μm; carrier gas, He 2.5 mL/min; injector and detector temperatures were, respectively, 280 and

Table 2. Results of HPLC-PDA/MS Screening Analysis of Extracts from Vegetative and Generative *Silphium* Organs

					percentage (±SD, $n = 5$) of particular components in extracts														
					S. perfoliatum					*S. trifoliatum*					*S. integrifolium*				
no.	compd	R_t (min)	λ_{max} (nm)	main ions of MS spectrum	L[a]	I	S	Rh	Ro	L	I	S	Rh	Ro	L	I	S	Rh	Ro
1	**1**	27.4	205	615 (100), 1171 (69), 585 (51)	1.50 ± 0.09	2.76 ± 0.23	21.86 ± 0.84	5.65 ± 0.44	3.45 ± 0.30		0.70 ± 0.11	6.87 ± 0.52	2.05 ± 0.12	1.50 ± 0.07	t	1.01 ± 0.06	3.04 ± 0.35	0.63 ± 0.05	0.49 ± 0.05
2	**2**	29.2	205	855 (100), 856 (36), 1153 (11)						t	0.44 ± 0.03	5.89 ± 0.38	1.15 ± 0.06	t	t				
3	**3**	31.6	220	614 (100), 1169 (57), 615 (24)	t	t	t	t	t	1.21 ± 0.13									
4	**4**	33.2	205	588 (100), 1118 (45), 1117 (26)														11.67 ± 1.05	16.03 ± 1.12
5	**5**	35.0	205	611 (100), 1163 (57), 1096 (47)											t		t	t	7.56 ± 0.92
6	**6**	35.3	205	1017 (100), 538 (49), 958 (13)						4.68 ± 0.35	1.46 ± 0.10	t				1.78 ± 0.03	3.67 ± 0.31		
7	**7**	35.6	205	611 (100), 507 (38), 1133 (31)											t			t	9.11 ± 0.83
8	**8**	36.2	205	1163 (100), 611 (91), 1133 (87)	t	t	t	t	t								t	12.64 ± 1.15	16.10 ± 1.49
9	**9**	36.5	205	956 (100), 507 (50), 1434 (35)														10.31 ± 0.87	t
10	**10**	36.8	205	1059 (100), 1060 (28), 559 (14)						5.13 ± 0.61	7.03 ± 0.68				t	2.89 ± 0.27			
11	**11**	37.4	205	613 (100), 1167 (41), 598 (25)	1.62 ± 0.12	3.12 ± 0.29	40.55 ± 1.57	t	5.52 ± 0.42	t	t	t	t	1.02 ± 0.03					
12	**12**	38.2	205	855 (100), 856 (36), 857 (9)						7.05 ± 0.56	2.08 ± 0.12	t							
13	**13**	38.8	205	955 (100), 956 (58), 1046 (55)					27.99 ± 1.24				t	12.89 ± 0.94				5.45 ± 0.44	10.33 ± 0.99
14	**14**	40.1	205	926 (100), 1001 (60), 927 (56)	1.74 ± 0.09	2.91 ± 0.18	t	t	t						6.89 ± 0.55	6.67 ± 0.73	t	t	t
15	**15**	40.4	205	1001 (100), 1002 (23), 530 (21)	t	10.23 ± 0.78	11.45 ± 0.76	14.89 ± 0.66	11.15 ± 0.63	14.26 ± 0.89	10.17 ± 0.85	6.44 ± 0.72	23.16 ± 1.65	9.56 ± 0.78	6.15 ± 0.68	8.90 ± 0.95	46.15 ± 2.18	32.56 ± 1.85	12.67 ± 1.12
16	**16**	41.5	215	971 (100), 972 (57), 1043 (31)	t	T	t	t	t	1.56 ± 0.09					21.67 ± 1.35	15.86 ± 1.04	21.83 ± 1.52		
17	**17**	42.0	205	1005 (100), 1006 (40), 1007 (16)	t	t	26.03 ± 1.03	t	t										
18	**18**[b]	42.5	205	793 (100), 794 (56), 875 (38)	2.53 ± 0.24	t	t	16.18 ± 0.71	6.21 ± 0.45				5.78 ± 0.41	12.45 ± 1.03	t	t	t	5.06 ± 0.48	4.34 ± 0.55
19	**19**	42.8	205	1043 (100), 1041 (41), 918 (31)	t	t	t	t	t	9.19 ± 0.48	13.07 ± 1.12				6.06 ± 0.50	8.22 ± 0.72			
20	**20**	43.0	205	793 (100), 1191 (67), 794 (53)	8.74 ± 0.55	16.45 ± 0.87			9.26 ± 0.54				8.78 ± 0.59	17.67 ± 1.52	t	t	t	9.52 ± 0.67	10.57 ± 0.81
21	**21**	43.2	205	1043 (100), 1044 (80), 919 (33)							12.14 ± 0.74								
22	**22**	43.4	205	841 (100), 842 (35), 817 (31)	t	t	t	t	t						6.86 ± 0.63	t	2.15 ± 0.23		
23	**23**	45.2	205	839 (100), 840 (56), 841 (14)	18.03 ± 0.82	8.01 ± 0.56	t	11.04 ± 0.52	8.45 ± 0.33	25.03 ± 1.28	11.33 ± 0.89	28.67 ± 1.65	33.00 ± 2.03	18.26 ± 1.28	28.17 ± 1.74	0.45 ± 0.01	7.00 ± 0.58	4.78 ± 0.58	4.34 ± 0.36
24	**24**	45.8	205	1043 (100), 551 (31), 1044 (26)	t	t	t	t	t	2.18 ± 0.18	4.75 ± 0.27	t	2.35 ± 0.09	t	1.05 ± 0.10	9.67 ± 0.85	9.42 ± 0.77		
25	**25**	47.3	205	897 (100), 898 (39), 899 (16)						1.54 ± 0.09	1.36 ± 0.05				1.89 ± 0.13	1.78 ± 0.07	t		
26	**26**	48.1	205	884 (100), 855 (57), 885 (41)											6.21 ± 0.72	10.50 ± 0.79	t		
27	**27**	48.6	205	1085 (100), 835 (32), 1086 (33)						0.87 ± 0.05	3.61 ± 0.29				t	4.67 ± 0.35			
28	**28**	50.9	210	881 (100), 882 (49), 884 (8)	1.34 ± 0.07	t	t	t	t	5.67 ± 0.48	6.45 ± 0.71	t	0.16 ± 0.01	1.78 ± 0.15	0.56 ± 0.01	1.12 ± 0.06	t		
29	**29**	51.5	210	793 (100), 794 (37), 1588 (23)				29.78 ± 1.01	15.18 ± 0.54					7.33 ± 0.59				3.23 ± 0.27	3.26 ± 0.25
30	**30**	51.8	210	881 (100), 882 (45), 883 (13)	1.87 ± 0.16	t	t	t	t	10.44 ± 0.92	3.16 ± 0.22	t	9.35 ± 0.69	3.15 ± 0.16	2.78 ± 0.12	2.75 ± 0.24	t	t	t
31	**31**	53.0	220	839 (100), 840 (40), 841 (13)	2.63 ± 0.14	9.79 ± 0.73	t	5.07 ± 0.39	5.41 ± 0.41	2.48 ± 0.18	5.67 ± 0.41	20.89 ± 1.23	3.31 ± 0.41	3.41 ± 0.21	2.45 ± 0.25	10.65 ± 0.97	6.45 ± 0.62	1.89 ± 0.09	2.45 ± 0.23
32	**32**	54.0	210	764 (100), 765 (38), 1529 (17)	11.41 ± 0.43	9.23 ± 0.59	t	t	t		1.01 ± 0.06				1.89 ± 0.07	0.45 ± 0.01	t	t	t
33	**33**	55.3	210	881 (100), 882 (51), 735 (35)	t	3.04 ± 0.29	t	t	t	1.57 ± 0.08	1.78 ± 0.05	0.10 ± 0.00			1.00 ± 0.07	6.05 ± 0.57			
34	**34**	58.7	210	881 (100), 882 (41), 883 (20)	t	t	t	t	t	1.67 ± 0.12		7.89 ± 0.62				4.74 ± 0.29	t		
35	glucuronide F	59.7	205	1263 (100), 631 (82), 1264 (64)	31.34 ± 1.69	28.02 ± 1.24	t	17.34 ± 0.86	7.05 ± 0.82	1.13 ± 0.05	3.56 ± 0.31	t	5.67 ± 0.46	7.56 ± 0.61	6.07 ± 0.82	1.23 ± 0.06	t	2.11 ± 0.25	2.47 ± 0.30
36	**35**	63.6	210	677 (100), 678 (25), 699 (12)	17.06e ± 0.92	6.12 ± 0.43	t	t	t	4.23 ± 0.31	9.67 ± 0.75	23.15 ± 1.15	4.56 ± 0.37	3.11 ± 0.26		t	t	t	t

[a] L, leaves; I, inflorescences; S, seeds; Rh, rhizomes; and Ro, roots. [b] Mass spectra and retention time correspond to glucuronide D_2 (see **Table 3**); t, trace (<0.1%).

Table 3. Results of HPLC-PDA/MS Screening Analysis of Extracts from Pot Marigold (*C. officinalis*) Flowers and American Ginseng (*P. quinquefolium*) Roots

no.	compd	R_t (min)	λ_{max} (nm)	MS spectra	percentage (± SD, $n = 5$) of particular components in extracts	identification	refs
				C. officinalis			
1	**36**	27.4	205	615 (100), 1171 (69), 585 (51)	1.60 ± 0.06		
2	glucuronide A	37.0	205	1678 (100), 1117[f] (73), 1677 (70)	29.75 ± 1.12	MS	*30*, *33*
3	glucuronide C	39.1	205	955[f] (100), 1046 (67), 1017 (48)	4.18 ± 0.22	MS	*30*, *33*
4	**37**	40.9	205	1001 (100), 1002 (68), 530 (32)	2.58 ± 0.11		
5	glucuronide D_2	42.5	205	793[f] (100), 794 (57), 875 (38)	20.19 ± 0.94	MS	*30*, *33*
6	**38**	45.2	205	839 (100), 840 (56), 841 (14)	1.63 ± 0.07		
7	**39**	46.3	205	1029 (100), 1030 (37), 544 (31)	1.86 ± 0.13		
8	glucuronide B	48.6	205	955[f] (100), 1434 (87), 1433 (41)	21.93 ± 0.85	MS	*30*, *33*
9	**40**	50.1	210	1001 (100), 1002 (47), 1083 (22)	2.44 ± 0.16		
10	glucuronide D	53.7	205	1589 (100), 1588 (77), 793[f] (52)	7.77 ± 0.33	MS	*30*, *33*
11	glucuronide F	59.7	205	1263 (100), 631[f] (78), 1264 (69)	6.07 ± 0.31	MS, R_t	*30*, *33*
				P. quinquefolium			
12	ginsenoside Rg_1	25.6	205	859[e] (100), 1005 (95), 860 (40)	2.65 ± 0.18	MS, R_t	*31*, *32*
13	ginsenoside Re	26.7	205	1005[e] (100), 1006 (45), 1478 (21)	20.59 ± 1.07	MS, R_t	*31*, *32*
14	**41**[a]	32.6	205	901[e] (100), 875 (78), 613 (72)	1.32 ± 0.11		
15	**42**[b]	34.5	205	859 (100), 845[e] (96), 613 (72)	1.30 ± 0.08		
16	ginsenoside Rb_1	37.4	205	1167[e] (100), 1168 (75), 613 (70)	27.57 ± 1.85	MS, R_t	*31*, *32*
17	ginsenoside Rc	38.5	205	1137[e] (100), 598 (61), 1138 (35)	14.83 ± 0.56	MS, R_t	*31*, *32*
18	ginsenoside Rb_2	39.5	205	1137[e] (100), 598 (73), 1138 (35)	11.75 ± 0.42	MS, R_t	*31*, *32*
19	**43**[c]	41.0	205	634 (100), 697[e] (67), 1208 (52)	0.15 ± 0.02		
20	ginsenoside Rd	42.1	205	1005[e] (100), 1892 (35), 1006 (34)	18.33 ± 1.33	MS, R_t	*31*, *32*
21	**44**[d]	42.6	205	793[f] (100), 794 (47), 875 (39)	1.51 ± 0.17		

[a] Mass spectra correspond to vina-ginsenoside R_1 (*31*). [b] Mass spectra correspond to majonoside R_2 (*31*). [c] Mass spectra correspond to ginsenoside Rh_1 (*31*). [d] Mass spectra and retention times correspond to glucuronide D_2. [e] $(M + AcO)^-$. [f] $(M)^-$.

180 °C; split ratio, 1:10; and inject volume, 1 μL. A temperature gradient was applied (240 °C for 1 min, then incremented by 20 °C/min to 320 °C); mass range, 100−870 Da. A comparison of differences between studied raw materials was made applying Duncan's test using SAS software.

RESULTS AND DISCUSSION

Tables 2 and **3** contain the list of results from HPLC-PDA/MS screening analyses for all tested saponin extracts. On the base of the achieved data, it was found that the total number of 36 substances occurred in all tested silphium extracts. Components marked as **1**, **15**, **23**, **24**, **28**, **30**, **31**, **35**, and glucuronide F were present in almost all of the tested silphium organs. It is worth mentioning that five substances occurring in extracts from marigold flowers were characterized by identical retention times and mass spectra as the same compounds identified in extracts from silphium: The MS of substance **1** in silphium corresponded to the MS of substance **36** in marigold (t_r = 27.4 min), **15→37** (t_r = 40.9 min), **18**→glucuronide D_2 (t_r = 42.5 min), **23→38** (t_r = 45.2 min), and glucuronide F (t_r = 59.7 min).

Six glucuronides of oleanolic acid (A, B, C, D, D_2, and F) and five unidentified substances with a domination of glucuronides A, B, and D_2 were found in the extract from marigold flowers. Glucuronides A, C, and D_2 are bisdesmosides, e.g., saponins with sugar chains (glucose or glucuronic acid) bonded to a C-3 hydroxyl group of oleanolic acid as well as a glucose moiety substituted instead by a hydrogen atom in the carboxylic function group. Glucuronides B, D, and F are monodesmosides (*33*). Ten saponin glycosides were identified in extracts prepared from ginseng roots: Rg_1, Re, Rb_1, Rc, Rb_2, and Rd and compounds **41**, **42**, **43**, and **44**. The MS of substance **41** corresponded to the MS of vina-ginsenoside R_1 (*31*), substance **42**→majonoside R_2 (*31*), and substance **43**→ginsenoside Rh_1 (*31*). The MS and retention time of substance **44** in ginseng corresponded to the MS and retention time of glucuronide D_2 in marigold, indicating that only the component of analyzed ginseng extract is the triterpene saponin of oleanolic acid.

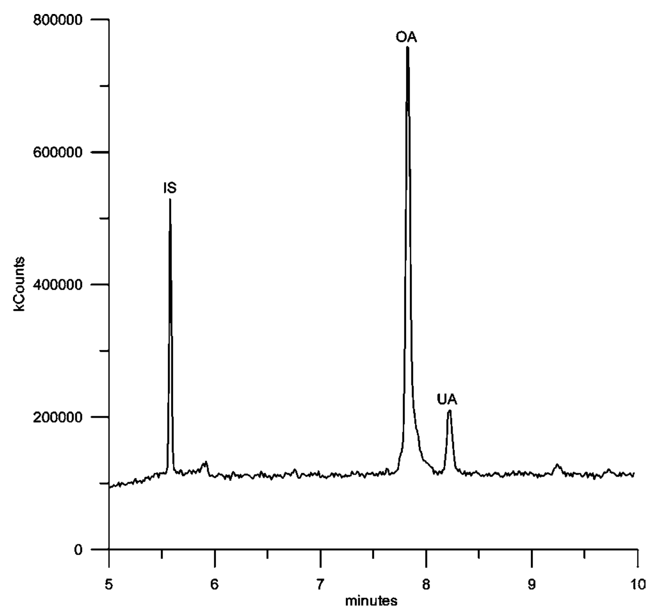

Figure 1. GC-MS chromatogram for silylo-derivative of saponin aglycones from *S. trifoliatum* inflorescences. Peak identification: IS, cholesterol as internal standard; OA, oleanolic acid; and UA, ursolic acid.

Figure 1 presents the example of GC-MS chromatogram for sililo-derivative of saponin aglycones from *S. trifoliatum*; **Figure 2** contains mass spectra of identified silylo-derivatives of oleanolic and ursolic acids. The analyses revealed that triterpene aglycones of saponins isolated from tested *Silphium* and *Calendula* species are oleanolic acid (t_r = 7.9 min) and ursolic acid (t_r = 8.3 min). Mass spectra for silylo-derivatives of oleanolic and ursolic acids show a great deal of similarity (**Figure 2**). Hederagenine was absent in aglycone fractions from

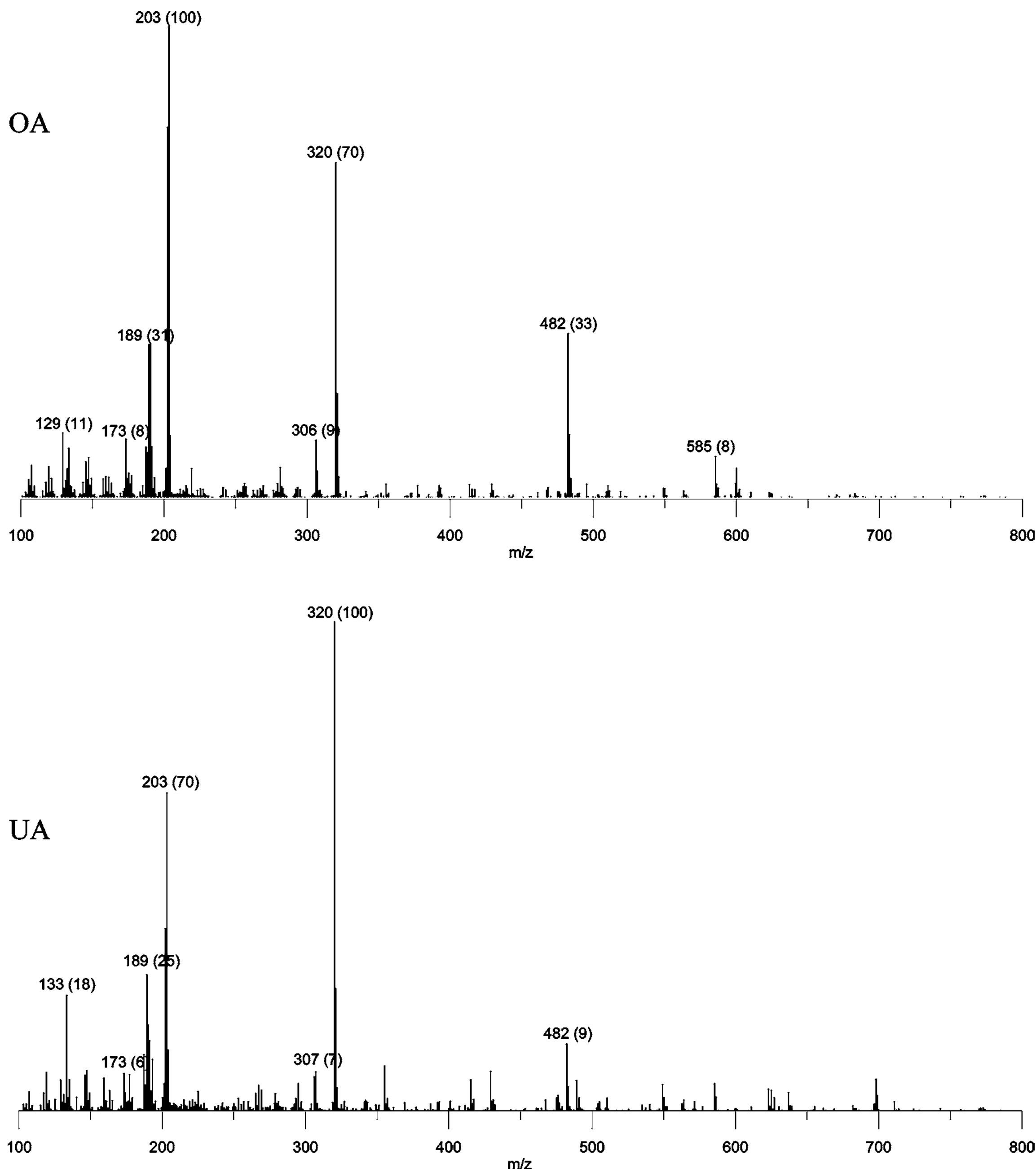

Figure 2. Mass spectra for silylo-derivatives of oleanolic acid (OA) and ursolic acid (UA).

silphium, marigold, and ginseng. It was found that ginseng roots contained only the aglycone of oleanolic acid within the triterpene saponin group. Detailed analysis of all of the achieved results for chemically and taxonomically distinct raw material allows for excluding the formation of triterpene artifacts during the hydrolysis of saponins isolated from tested materials. No proof of forming the artifacts or isomers or analogues by oleanolic acid, ursolic acid, and hederagenine under the same conditions as during hydrolysis was found either.

The quantitative analyses revealed that referring to saponin aglycones, particular tested silphium organs significantly differed. **Figure 3** presents the results of quantitative analyses for particular triterpene saponin aglycones present in silphium, marigold, and ginseng saponins. The leaves of silphium harvested in May were characterized by the highest content of oleanolic acid—They contained 17.03 mg/g dry weight of the triterpenic acid, on average (from 10.15 mg/g in *S. integrifolium* up to 22.08 mg/g in *S. trifoliatum*). The dynamics of oleanolic acid content changes indicates that in general its concentration in the leaves decreases along with plant development. A similar dependence was earlier observed by other authors (*19*). Moreover, it was found that inflorescences of *S. trifoliatum* and *S. integrifolium* contained oleanolic acid in amounts of 22.05 mg/g dry weight and 17.95 mg/g dry weight, respectively, whereas *S. perfoliatum* inflorescences contained much lower concentrations—3.68 mg/g dry weight. When comparing the oleanolic acid composition of marigold flowers (20.53 mg/g dry weight) with results achieved for *S. trifoliatum* and *S. integrifolium* inflorescences, it is obvious that *Silphium* species may compete with the former. Furthermore, oleanolic acid amounts found in silphium leaves are also the promising source of oleanolic acid glycosides or even aglycone itself that is liberated from those glycosides due to hydrolysis. When comparing the oleanolic acid content in silphium and marigold, the biomass production should also be taken into account, which is high in the case of silphium, and at the beginning of *S. perfoliatum* flowering, it

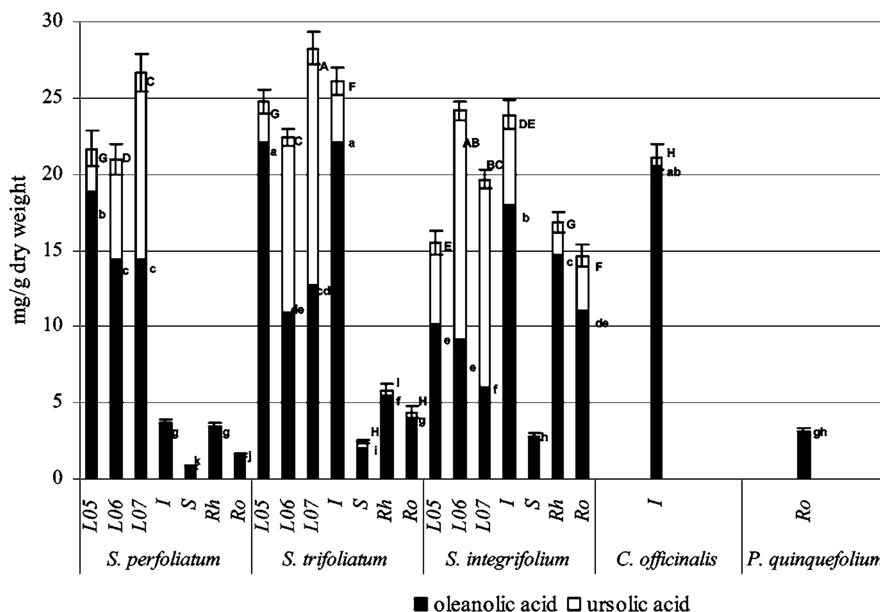

Figure 3. Contents of glycoside-bonded oleanolic and ursolic acids (±SD, $n = 5$) in *S. perfoliatum*, *S. trifoliatum*, *S. integrifolium*, *C. officinalis*, and *P. quinquefolium*. Key: L, leaves; I, inflorescences; S, seeds; Rh, rhizomes; Ro, roots; 05, May; 06, June; and 07, July. Values designated for oleanolic acid with the same letters (a, b, c, etc.) do not significantly differ at 5% error. Values for ursolic acid designated with the same letters (A, B, C, etc.) do not significantly differ at 5% error.

amounts to from 305 to 770 g of fresh material of basal shoots (*25*). Statistical analysis (**Figure 3**) of oleanolic acid content results in tested organs of silphium confirmed that leaves of *S. perfoliatum* and *S. trifoliatum* collected at the plant intensive growth stage (May) and inflorescences of *S. integrifolium* and *S. trifoliatum* may be an alternative source of these compounds as compared to well-known marigold.

Contents of oleanolic acid in roots and rhizomes of silphium are at the level of from 1.55 mg/g dry weight in *S. perfoliatum* roots to 14.70 mg/g dry weight in *S. integrifolium* rhizomes. Seeds contain from 0.75 mg/g dry weight of the acid in *S. perfoliatum* to 2.78 mg/g dry weight in *S. integrifolium*. However, one should bear in mind that the saponin glycoside presence in seeds may affect the decrease of the germination ability of these species (*34*).

The oleanolic acid content in ginseng roots was 3.15 mg/g dry weight, which as compared to other materials was not a significant source of the compound. Ursolic acid, which most abundantly occurs in *S. integrifolium* and *S. trifoliatum* at a concentration of about 14.98 mg/g dry weight in leaves harvested before flowering (June) and to 15.50 mg/g dry weight in leaves collected during flowering, is another saponin aglycone identified in the studied raw material. No ursolic acid was found in ginseng roots. The analysis of the triterpenic acid content dynamics in bonded forms reveals its increase in leaves collected in June before the flowering stage as compared to material harvested in May. It should be underlined that ursolic acid identification as an aglycone of triterpene glycosides from silphium is the first report in that branch.

When summarizing the aglycone triterpene fraction, the following conclusion may be drawn. Leaves of three *Silphium* species, inflorescences of *S. trifoliatum* and *S. integrifolium*, as well as underground organs of *S. integrifolium* are the most abundant in this group of compounds. The seasons before flowering and at the beginning of that stage appear to be the most efficient periods for leaf collection in reference to triterpene aglycones contents in plant yield. Phytochemical studies indicate that tested materials may be an alternative source of triterpene saponins and their aglycones as compared to commonly known pot marigold flowers.

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