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COMPONENTS OF THE PETROLEUM ETHER AND CHLOROFORM EXTRACTS OF *Chrysosplenium alternifolium*

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Chrysosplenium alternifolium L. (Saxifragaceae), golden saxifrage, is one of three species of the genus *Chrysosplenium* L. that occur often in Poland. *Ch. alternifolium* is a perennial herb that widely inhabits moist or wet soils and that prefers a shady position, thriving in woodland. The plant is found mainly in waste, damp, wet places on lowlands up to the lower mountains passage [1].

It is used in the symptomic treatment of digestive disorders related to liver and spleen activities. It is also used in the treatment of skin diseases [2].

According to the literature, little is known about the chemical composition of *Ch. alternifolium.* Earlier phytochemical studies of golden saxifrage proved the presence of arbutin and undefinable tannins. The chromatographic analysis after acid hydrolysis revealed the presence of flavonoid compounds [3, 4].

The chemical composition of petroleum ether and chloroform extracts from *Chrysosplenium alternifolium* herb has been studied. From the petroleum ether extract after column chromatographic separation, steroidic fraction S and flavonoid compound **1** were obtained. From the chloroform extract using multistep column chromatography the next three crystalline homogeneous flavonoid compounds **2**–**4** were isolated.

The steroidic fraction S was investigated by GC and GC/MS techniques, and four steroidic compounds were identified: β-sitosterol (major), campesterol, α-saccharostenone, and stigmasterol (traces) (Table 1).

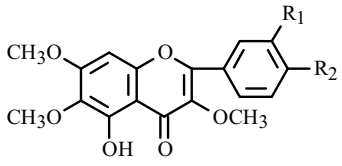
The structures of flavonoid compounds **1**–**4** were proved by spectral data from ^{1}H NMR and UV spectroscopy.

Compound **1** was identified as 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone (chrysosplenol B), and compound **2** as 5,3′,4′-trihydroxy-3,6,7-trimethoxyflavone (chrysosplenol D).

Compound **3** upon acid hydrolysis yields glucose and aglycone identical with compound **1**. Compound **4** yields also glucose and aglycone identical with compound **2**. The glucose was connected to C-4′ of the aglycone **3** and **4**, as proved by UV spectral analysis.

Compound **3** was identified as 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone 4′-*O*-β-D-glucopyranoside (chrysosplenoside B) and compound **4** as 5,3′,4′-trihydroxy-3,6,7-trimethoxyflavone 4′-*O*-β-D-glucopyranoside (chrysosplenoside D) by means of ^{1}H NMR analysis. In both cases, signals of the anomeric proton of glucose were observed (the coupling constants characteristic for the β-configuration).

Chrysosplenols B and D were identified earlier after acid hydrolysis of *Chrysosplenium alternifolium* [4]. Chrysosplenosides B and D were isolated from *Chrysosplenium japonicum* [5].



1 - 4

1: $R_1 = OCH_3$, $R_2 = OH$; **2:** $R_1 = R_2 = OH$
3: $R_1 = OCH_3$, $R_2 = O$-Glc; **4:** $R_1 = OH$, $R_2 = O$-Glc

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TABLE 1. GC/MS Data of Identified Compounds from Steroidic Fraction S

Compound	M^+, *m/z*	Rt, min	Compound	M^+, *m/z*	Rt, min
Dibutyl phthalate	278	4.07	Campesterol	400	25.23
Diisoctyl phthalate	390	15.75	Stigmasterol	412	25.67
Ethyl ester of lignoceric acid	396	19.23	β-Sitosterol	414	26.28
Ethyl ester of hexacosanoic acid	424	23.17	α-Saccharostenone	410	27.76

In biological tests, chrysosplenol B showed potent antiviral activity and chrysosplenol D possessed some antitumor activity [6, 7].

Plant Material. *Ch. alternifolium* herb was collected at the end of April 2005 near Bialystok and Lodz (Poland). A voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Lodz. The material was dried at room temperature (490 g).

Chromatography. Column chromatography (CC): MN-Kieselgel 60 (70–270 mesh ASTM); polyamide (Roth). Thin layer chromatography (TLC): plates cellulose precoated (Merck) – TLC_c; plates silica gel 60 precoated (Merck) – TLC_g; plates polyamide $11F_{254}$ precoated (Merck) – TLC_p.

Solvent systems: S_1 HOAc-conc. HCl–H_2O (30:3:10); S_2 ethyl acetate–methanol (9:1), S_3 *n*-butanol–pyridine–H_2O (6:4:3). The spots of sterols were visualized by spraying with Liberman–Burchard reagent and heating at 105°C. The flavonoids were visualized under UV light (366 nm) before and after spraying with 2% $AlCl_3$ in methanol and 0.5% NA-reagent in methanol. Sugars were visualized by spraying with aniline phthalate and heating at 105°C.

Extraction and Separation. Dried and powdered *Ch. alternifolium* herb (490 g) was successively extracted with petroleum ether and chloroform in a Soxhlet apparatus. After evaporation of solvents under reduced pressure, 18.5 g of waxy residue (from petroleum ether extract) and 11 g of similar waxy residue (from chloroform extract) were obtained.

The first residue (18.5 g) was separated by column chromatography over silica gel (430 g) with successive elution by petroleum ether, petroleum ether–benzene (gradient), benzene, benzene–chloroform (gradient), chloroform, chloroform–ethyl acetate (gradient), ethyl acetate, and ethyl acetate–methanol (gradient). The composition of fractions was analyzed by TLC. Elution by petroleum ether–benzene (1:9) afforded steroidic fraction S (300 mg). Fraction S after crystallization from EtOH was analyzed by GC and GC-MS. Elution by chloroform–ethyl acetate (8:2) afforded compound **1** (80 mg).

The second residue (11 g) was separated by column chromatography over silica gel (350 g) with successive elution by petroleum ether, petroleum ether–benzene (gradient), benzene, benzene–chloroform (gradient), chloroform, chloroform–ethyl acetate (gradient), ethyl acetate, and ethyl acetate–methanol (gradient). The composition of fractions was analyzed by TLC.

Compound **2** (160 mg) was eluted with chloroform–ethyl acetate (7:3) as a solvent system.

Compound **3** (500 mg) was eluted with ethyl acetate–methanol (9:1) in further fractions. The first fractions eluted with ethyl acetate–methanol (9:1) were separated by column chromatography over polyamide (28 g) with successive elution by ethyl acetate and ethyl acetate–methanol (gradient).

Compound **4** (350 mg) was obtained from fraction eluted with ethyl acetate–methanol (9:1).

GC Analysis was carried out using a Carlo Erba chromatograph HRGC 5300 Mega series equipped with an FID detector, injector SSL, and HP-5 capillary column (30 m in length and 0.32 mm bore, film thickness 0.25 μm). The oven temperature was programmed from 200 to 320°C (30 min isothermic), growth of temperature 6°/min, temperature of injector 330°C, temperature of detector 330°C, flow-rate of carrier gas (N_2) 2.0 mL/min.

GC-MS Analysis was performed on GC 8000 Fisons Instruments combined with an MD 800 mass spectrometer. The column and conditions were the same as in the case of GC. The flow-rate of the carrier gas (He) was 1.5 mL/min, the ion source temperature was 200°C, and the electron impact energy was 70 eV. The identification of the compounds of the steroidic fraction S was based on a comparison of retention times and mass spectra with those of authentic samples and with the NIST MS Library (Table 1).

Identifications of Flavonoids. Melting points (mp), uncorrected, were determined on a Boetius apparatus. Flavonoids were identified by chromatographic analysis of acid hydrolysates and by spectroscopic methods. Total acid hydrolysis was carried out with 5% HCl for 3 h under reflux. Ethyl acetate extracts of hydrolysates were analyzed for aglycones (TLC_c, S_1), and H_2O residues for sugars (TLC_c, S_3). The UV spectra (specord 40 UV-VIS) of flavonoids were recorded in (a) MeOH, also after the addition of (b) NaOMe, (c) $AlCl_3$, (d) $AlCl_3$–HCl, (e) NaOAc, and (f) NaOAc–H_3BO_3 according to Mabry et al. [8].

^{1}H NMR spectra were recorded on a DRX 500 at 500.13 MHz (TMS as an internal standard).

Compound 1, $C_{19}H_{18}O_8$; pale yellow needles; mp 182–184°C; R_f 0.92 (TLC_c-S_1); UV spectrum (MeOH, λ_{max}, nm): a) 257, 271, 351; b) 266, 294 sh, 414; c) 268, 301 sh, 382, 399 sh; d) 267 sh, 281, 376, 400 sh; e) 257, 271, 351; f) 257, 270, 350; ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.63 (1H, s, 5-OH), 9.97 (1H, br.s, 4′-OH), 7.67 (1H, br.s, H-2′), 7.63 (1H, br.d, J = 8.5, H-6′), 6.96 (1H, d, J = 8.4, H-5′), 6.93 (1H, s, H-8), 3.92 (3H, s, 7-OCH_3), 3.86 (3H, s, 3′-OCH_3), 3.81 (3H, s, 3-OCH_3), 3.72 (3H, s, 6-OCH_3).

Compound 2, $C_{18}H_{16}O_8$; pale yellow needles; mp 240–242°C; R_f 0.89 (TLC_c-S_1); UV spectrum (MeOH, λ_{max}, nm): a) 258, 270, 353; b) 268, 291 sh, 407; c) 278, 304 sh, 439; d) 272, 298 sh, 380, 405 sh; e) 260, 272, 355; f) 264, 285 sh, 380; ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.65 (1H, s, 5-OH), 9.81 (1H, s, 4′-OH), 9.37 (1H, s, 3′-OH), 7.59 (1H, d, J = 1.6, H-2′), 7.48 (1H, dd, J_1 = 1.5, J_2 = 8.5, H-6′), 6.90 (1H, d, J = 8.5, H-5′), 6.85 (1H, s, H-8), 3.91 (3H, s, 7-OCH_3), 3.79 (3H, s, 3-OCH_3), 3.72 (3H, s, 6-OCH_3).

Compound 3, $C_{25}H_{28}O_{13}$; pale yellow needles; mp 222–224°C; R_f 0.58 (TLC_p-S_2); UV spectrum (MeOH, λ_{max}, nm): a) 253, 273, 340; b) 292, 329 sh; c) 256 sh 273, 369, 426 sh; d) 261 sh, 285, 360, 426 sh; e) 253, 272, 340; f) 253, 273, 341; ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.56 (1H, s, 5-OH), 7.70–7.68 (2H, m, H-2′,6′), 7.27 (1H, d, J = 9.1, H-5′), 6.97 (1H, s, H-8), 5.06 (1H, d, J = 7.0, H-1″), 3.92 (3H, s, 7-OCH_3), 3.87 (3H, s, 3′-OCH_3), 3.82 (3H, s, 3-OCH_3), 3.72 (3H, s, 6-OCH_3).

Compound 4, $C_{24}H_{26}O_{13}$, pale yellow needles; mp 163–165°C; R_f 0.27 (TLC_p-S_2); UV spectrum (MeOH, λ_{max}, nm): a) 254, 272, 340; b) 268, 329 sh, 359 sh; c) 271, 296 sh, 362, 405 sh; d) 284, 362, 405 sh; e) 254, 270, 341; f) 254, 272, 342; ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 12.55 (1H, s, 5-OH), 9.07 (1H, s, 3′-OH), 7.60 (1H, d, J = 1.8, H-2′), 7.56 (1H, dd, J_1 = 1.9, J_2 = 8.5, H-6′), 7.25 (1H, d, J = 8.7, H-5′), 6.93 (1H, s, H-8), 4.88 (1H, d, J = 7.2, H-1″), 3.90 (3H, s, 7-OCH_3), 3.80 (3H, s, 3-OCH_3), 3.72 (3H, s, 6-OCH_3).

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