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*Supplementary materials*

# Khellactone Derivatives and Other Phenolics of *Phlojodicarpus sibiricus* (Apiaceae): HPLC-DAD-ESI-QQQ-MS/MS and HPLC-UV Profile, and Antiobesity Potential of Dihydrosamidin

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**Abstract:** With obesity, the consumption of phenolic-enriched food additives as a part of traditional nutrition avoids the negative implications of eating high-calorie products. This study investigated the new herbal food additive, *Phlojodicarpus sibiricus* roots and herb, ubiquitously used in Siberia as a spice. Chromatographic techniques such as HPLC-DAD-ESI-QQQ-MS/MS and microcolumn HPLC-UV were the basic instruments for component profiling and quantification, and antiobesity potential was investigated using a differentiated 3T3-L1 adipocytes assay. We found that the roots and herb of *P. sibiricus* were high-coumarin-containing additives inhibiting triacylglycerol accumulation in 3T3-L1 preadipocytes. Forty-one phenolics were detected in *P. sibiricus* extracts, and 35 were coumarins, including 27 khellactone derivatives present as esters and glucosides. Total coumarin content varied from 36.16 mg/g of herb to 98.24 mg/g of roots, and from 0.32 mg/mL to 52.91 mg/mL in *P. sibiricus* preparations. Moreover, Siberian populations of *P. sibiricus* were characterised by a different HPLC-based coumarin profile. The most pronounced inhibiting effect on triacylglycerol accumulation in 3T3-L1 preadipocytes was shown for dihydrosamidin (khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester), which was more active than other khellactone esters and glucosides. The results demonstrated that if used as a food additive *Phlojodicarpus sibiricus* could be a source of bioactive coumarins of the khellactone group with high antiobesity potential.

**Keywords:** *Phlojodicarpus sibiricus*; khellactone esters; dihydrosamidin; HPLC-MS; antiobesity activity; 3T3-L1 adipocytes.

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**Table S1.** Coumarins found in *Phlojodicarpus* genus.

| Compound | *P. sibiricus* | | *P. turczaninovii* | | *P. villosus* | |
|---|---|---|---|---|---|---|
| | **Roots** | **Herb** | **Roots** | **Herb** | **Roots** | **Herb** |
| *Simple coumarins* | | | | | | |
| Umbelliferone | [8] | | | [13] | | |
| Umbelliferone 7-*O*-(6′-Api*f*)-Glc*p* (6′-apiosyl-skimmin) | | [12] | | | | [11] |
| Peucedanol 3-*O*-Glc | | | | [14] | | |
| Phlojodicarpin | | [10] | | | | |
| Isophlojodicarpin | | [10] | | | | |
| 7-Hydroxy-8-(2',3'-dihydroxy-3'-methylbutyl)-coumarin 7-O-Glc*p* | | [11] | | | | |
| Scopoletin | [8] | | | [13] | | |
| *Furocoumarins* | | | | | | |
| Isoimperatorin | | [12] | | | | |
| Marmesin | | | | [14] | | |
| Angelicin | | | [13] | | | |
| Columbianetin (zosimol) | | | [13] | [13] | | |
| Columbianetin 10-*O*-Sen (libanorin) | | | [13] | [14] | | |
| Columbianetin 10-*O*-Ang (columbianadin, zosimin) | | | [13] | [13] | | |
| Oroselone | | | | [13] | | |
| Oroselol | | | [13] | [13] | | |
| 8,9-Dihydrooroselol (vaginidiol) | | | [13] | | | |
| 8,9-Dihydrooroselol 8-*O*-iVal | | | [13] | | | |
| 8,9-Dihydrooroselol 8-*O*-mBu | | | [13] | [13] | | |
| 8,9-Dihydrooroselol 8-*O*-iVal-9-*O*-mBu | | | [13] | | | |
| 8,9-Dihydrooroselol 9-*O*-Ac-10-*O*-Sen (peucenidin) | | | [13] | [14] | | |
| 8,9-Dihydrooroselol 9-*O*-Ang-10-*O*-Ac (libanotin, edultin) | | | [13] | [13] | | |
| *Pyranocoumarins* | | | | | | |
| Decursinol | | | | [14] | | [15] |
| Decursinol 3-*O*-Sen (decursin) | | | | | | [15] |
| Decursinol 3-*O*-Ang (agasyllin) | | | | | | [15] |
| Lomatin 3′-*O*-Sen (buchtormin, nuttallin) | | | | [14] | | |
| Dihydrolomatin | | | | | | |
| Khellactone 4'-*O*-Me | | [16] | | | | |

| | | | |
|---|---|---|---|
| Khellactone 3′-*O*-mBu-4′-*O*-Ac (visnadin) | [6] | | [6] |
| Khellactone 3′-*O*-iVal-4′-*O*-Ac (dihydrosamidin) | [7] | | [7] |
| Khellactone 3′-*O*-Ac-4′-*O*-iVal (suksdorfin) | | [16] | |
| Khellactone 3'-*O*-Ac-4'-*O*-mBu | | [16] | |

Ac – acetyl, Ang – angeloyl, Api*f* – apiosylfuranose, Glc*p* – glucosylpyranose, iVal – isovaleroyl, mBu – 2-methylbutanoyl, Me – methyl, Sen – senecioyl.

**Table S2.** Triacylglycerol (TG) content in 3T3-L1 adipocytes after incubation with *P. sibiricus* root fractions [a, b]

| Fraction | TG, μg/mg protein [c] |
|---|---|
| Hexane fraction | 316.2 ± 12.8* |
| Water fraction | 627.1 ± 25.1* |
| Essential oil | 783.7 ± 35.2 |
| Water-soluble polysaccharide | 815.3 ± 35.6 |
| Pectic substances | 809.6 ± 38.0 |
| 5-*O*-Caffeoylquinic acid (reference compound) | 286.2 ± 11.4* |
| Control (water) | 812.8 ± 25.1 |

[a] Averages ± standard deviation were obtained from four different experiments. [b] Concentration used 50 μg/mL. [c] Values with asterisk (*) indicate statistically significant differences with the control groups at $p < 0.05$ by one-way ANOVA.

**Table S3.** Essential oil composition (percentage of total component content) of *P. sibiricus* roots.

| Compound | RI | MI [a] | % |
|---|---|---|---|
| α-Pinene | 932 | i, ii, iii | 1.5 |
| Sabinene | 973 | i, ii, iii | 40.3 |
| β-Pinene | 975 | i, ii, iii | 3.2 |
| β-Myrcene | 991 | i, ii, iii | 0.2 |
| Δ-Carene | 1011 | i, ii, iii | 2.6 |
| *p*-Cymene | 1024 | i, ii, iii | 1.5 |
| β-Phellandrene | 1027 | i, ii, iii | 0.2 |
| Limonene | 1029 | i, ii, iii | 30.4 |
| 1,8-Cineol | 1031 | i, ii, iii | 0.1 |
| γ-Terpinene | 1058 | i, ii, iii | 0.1 |
| Terpinolene | 1089 | i, ii, iii | 1.9 |
| Linalool | 1100 | i, ii, iii | 0.5 |
| Terpinene-4-ol | 1177 | i, ii, iii | 5.7 |
| Thymol | 1292 | i, ii, iii | 2.7 |
| Carvacrol | 1302 | i, ii, iii | 0.9 |
| α-Copaene | 1377 | i, ii | 0.1 |
| β-Elemene | 1392 | i, ii, iii | 1.2 |
| β-Caryophyllene | 1420 | i, ii, iii | 0.1 |
| Aromadendrene | 1441 | i, ii, iii | 1.2 |
| α-Humulene | 1456 | i, ii, iii | 0.1 |
| β-Selinene | 1488 | i, ii | 1.9 |
| α-Selinene | 1497 | i, ii | 3.3 |
| δ-Cadinene | 1527 | i, ii, iii | 0.1 |
| Caryophyllene oxide | 1587 | i, ii, iii | 0.1 |
| Total | | | 99.9 |

[a] Methods of identification: i—retention index, ii—mass spectrum, iii—co-injection with authentic sample.

**Table S4.** Monosaccharide composition of crude water-soluble polysaccharide fraction (WSPS) and crude pectic substances fraction (PS) from *P. sibiricus* roots, mol%.

| Monosaccharide | WSPS | PS |
|---|---|---|
| Arabinose | 1.5 | 5.1 |
| Fucose | Traces | Traces |
| Galactose | 25.8 | 7.6 |
| Glucose | 59.4 | 5.1 |
| Mannose | 1.4 | Traces |
| Rhamnose | 1.9 | 15.4 |
| Xylose | Traces | Traces |
| Galacturonic acid | 8.3 | 46.2 |
| Glucuronic acid | 1.6 | 20.5 |

**Table S5.** Retention times ($t_R$), peak asymmetry factors (AsF), and theoretical plate number (N) for 8 compounds and internal standard (I.S.).

| Compound | $t_R$, min | AsF | N |
|---|---|---|---|
| 9 | 15.53 | 1.02 | 61256 ± 1102 |
| 11 | 18.86 | 0.99 | 75544 ± 1208 |
| 12 | 19.32 | 1.04 | 83427 ± 1668 |
| 15 | 20.18 | 1.01 | 105894 ± 1482 |
| 17 | 20.76 | 0.98 | 127610 ± 2041 |
| 27 | 5.78 | 0.97 | 36866 ± 737 |
| 31 | 7.85 | 1.02 | 40075 ± 761 |
| 36 | 6.22 | 1.03 | 38325 ± 804 |
| I.S. | 14.35 | 1.01 | 73778 ± 1475 |

[a] Compounds: **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid, I.S. – internal standard (pimpinellin).

**Table S6.** Regression equations, correlation coefficients ($r^2$), standard deviation ($S_{YX}$), limits of detection (LOD), limits of quantification (LOQ) and linear ranges for 8 compounds.

| Compound | Regression equation | $r^2$ | $S_{YX}$ | LOQ (μg/mL) | LOD (μg/mL) | Linear range (μg/mL) |
|---|---|---|---|---|---|---|
| 9 | $y = 0.039 \cdot x - 0.007$ | 0.9999 | $8.07 \cdot 10^{-3}$ | 0.68 | 2.05 | 2.5–1000.0 |
| 11 | $y = 0.040 \cdot x - 0.001$ | 0.9999 | $1.04 \cdot 10^{-2}$ | 0.83 | 2.50 | 3.0–1000.0 |
| 12 | $y = 0.037 \cdot x - 0.008$ | 0.9999 | $6.18 \cdot 10^{-3}$ | 0.53 | 1.62 | 2.0–1000.0 |
| 15 | $y = 0.033 \cdot x - 0.010$ | 0.9999 | $8.18 \cdot 10^{-3}$ | 0.80 | 2.42 | 2.5–1000.0 |
| 17 | $y = 0.032 \cdot x - 0.007$ | 0.9999 | $9.16 \cdot 10^{-3}$ | 0.93 | 2.81 | 3.0–1000.0 |
| 27 | $y = 0.032 \cdot x - 0.003$ | 0.9999 | $7.49 \cdot 10^{-3}$ | 0.72 | 2.18 | 2.5–1000.0 |
| 31 | $y = 0.030 \cdot x - 0.010$ | 0.9999 | $8.24 \cdot 10^{-3}$ | 0.88 | 2.67 | 3.0–1000.0 |
| 36 | $y = 0.057 \cdot x - 0.001$ | 0.9999 | $8.59 \cdot 10^{-3}$ | 0.46 | 1.40 | 2.0–500.0 |

[a] Compounds: **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid.

**Table S7.** Intra- and inter-day precision, repeatability, stability and recovery for 8 compounds.

| Compound | Precision intra-day (RSD%) $n = 5$ | Precision inter-day (RSD%) $n = 4$ | Repeatability (RSD%) $n = 7$ | Stability (RSD%) $n = 7$ | Recovery (%) $n = 5$ |
|---|---|---|---|---|---|
| 9 | 1.85 | 2.46 | 1.93 | 2.11 | 97.34 |
| 11 | 1.35 | 1.62 | 1.87 | 1.57 | 100.29 |
| 12 | 1.53 | 2.29 | 2.07 | 1.73 | 98.22 |
| 15 | 1.12 | 1.50 | 1.45 | 1.40 | 99.32 |
| 17 | 1.27 | 1.94 | 2.16 | 2.25 | 102.64 |
| 27 | 0.97 | 1.26 | 1.21 | 1.52 | 100.07 |
| 31 | 2.06 | 2.57 | 2.59 | 2.86 | 101.39 |
| 36 | 1.62 | 2.02 | 1.57 | 2.29 | 101.25 |

[a] Compounds: **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid.

**Table S8.** Content of 8 compounds and sum of coumarins (ΣCou) in water-methanol mixtures (WM, *v*/*v*, %) media after extraction of *Phlojodicarpus sibiricus* roots and herb, μg/mL ± S.D.

| **Compound** [a] | **WM 100:0** | **WM 80:20** | **WM 60:40** | **WM 40:60** | **WM 20:80** | **WM 0:100** |
|---|---|---|---|---|---|---|
| | | | *P. sibiricus* roots | | | |
| 9 | Tr. | 32.94 ± 0.65 | 64.56 ± 1.28 | 83.17 ± 1.74 | 95.18 ± 1.90 | 85.44 ± 1.69 |
| 11 | Tr. | 25.94 ± 0.49 | 83.67 ± 1.67 | 155.54 ± 2.94 | 166.22 ± 3.25 | 162.08 ± 3.24 |
| 12 | Tr. | Tr. | Tr. | 47.73 ± 0.95 | 52.38 ± 1.04 | 49.45 ± 0.97 |
| 15 | 30.19 ± 0.60 | 370.44 ± 7.41 | 1537.05 ± 30.74 | 2644.33 ± 44.95 | 2825.09 ± 56.50 | 2742.51 ± 49.35 |
| 17 | Tr. | Tr. | Tr. | 17.41 ± 0.31 | 18.75 ± 0.35 | 17.44 ± 0.34 |
| 27 | 234.22 ± 3.98 | 238.52 ± 4.05 | 241.09 ± 4.58 | 250.53 ± 5.01 | 252.64 ± 5.06 | 250.99 ± 5.00 |
| 31 | Tr. | Tr. | Tr. | 42.76 ± 0.85 | 46.38 ± 0.87 | 45.64 ± 0.88 |
| 36 | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. |
| ΣCou | 264.41 | 667.84 | 1926.37 | 3176.33 | 3456.64 | 3353.55 |
| | | | *P. sibiricus* herb | | | |
| 9 | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. |
| 11 | 3.91 ± 0.07 | 69.02 ± 1.24 | 256.62 ± 4.86 | 368.82 ± 6.25 | 378.19 ± 7.18 | 359.56 ± 6.82 |
| 12 | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. |
| 15 | 7.22 ± 0.14 | 130.79 ± 2.35 | 346.36 ± 7.26 | 406.50 ± 7.71 | 412.92 ± 8.24 | 392.03 ± 7.44 |
| 17 | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. |
| 27 | 32.29 ± 0.64 | 52.23 ± 0.99 | 72.67 ± 1.51 | 72.83 ± 1.57 | 73.10 ± 1.55 | 52.18 ± 1.02 |
| 31 | 271.00 ± 4.87 | 282.78 ± 5.64 | 288.52 ± 5.76 | 288.84 ± 5.48 | 289.14 ± 5.49 | 110.21 ± 2.09 |
| 36 | 65.74 ± 1.42 | 125.72 ± 2.51 | 154.29 ± 2.92 | 154.82 ± 2.29 | 151.95 ± 2.88 | 58.94 ± 1.17 |
| ΣCou | 314.42 | 534.82 | 964.17 | 1136.99 | 1153.35 | 913.98 |

[a] Compounds: **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid. Tr. – traces. Extraction conditions: 400 mg of plant material was extracted by 10 mL of water-methanol mixtures in ultrasonic bath at 50°C for 30 min and after centrifuging and filtering was analyzed in mc-HPC-UV.

**Table S9.** Content of 8 compounds and sum of coumarins (ΣCou) in water-methanol (20:80, *v*/*v*) media after extraction of *P. sibiricus* roots and herb by various type of extraction [a], μg/mL ± S.D.

| Compound [b] | USE | MWAE | BWBE | RTE |
|---|---|---|---|---|
| | | *P. sibiricus* roots | | |
| 9 | 95.18 ± 1.90 | 90.34 ± 1.80 | 83.16 ± 1.16 | 37.57 ± 0.75 |
| 11 | 166.22 ± 3.25 | 147.12 ± 2.64 | 125.11 ± 2.62 | 96.30 ± 2.01 |
| 12 | 52.38 ± 1.04 | 42.64 ± 0.85 | 38.63 ± 0.79 | 10.83 ± 0.18 |
| 15 | 2825.09 ± 56.50 | 2637.22 ± 47.46 | 2230.54 ± 40.14 | 1437.21 ± 28.74 |
| 17 | 18.75 ± 0.35 | 16.37 ± 0.27 | 10.84 ± 0.21 | 4.32 ± 0.08 |
| 27 | 252.64 ± 5.06 | 244.41 ± 3.91 | 240.31 ± 4.32 | 121.65 ± 2.55 |
| 31 | 46.38 ± 0.87 | 42.39 ± 0.85 | 38.10 ± 0.64 | 20.63 ± 0.43 |
| 36 | Tr. | Tr. | Tr. | Tr. |
| ΣCou | 3456.64 | 3177.85 | 2766.69 | 1728.51 |
| | | *P. sibiricus* herb | | |
| 9 | Tr. | Tr. | Tr. | Tr. |
| 11 | 378.19 ± 7.18 | 352.41 ± 7.04 | 308.22 ± 5.85 | 243.25 ± 4.86 |
| 12 | Tr. | Tr. | Tr. | Tr. |
| 15 | 412.92 ± 8.24 | 401.03 ± 7.21 | 384.15 ± 6.52 | 202.76 ± 3.63 |
| 17 | Tr. | Tr. | Tr. | Tr. |
| 27 | 73.10 ± 1.55 | 64.18 ± 1.28 | 53.62 ± 1.01 | 27.04 ± 0.48 |
| 31 | 289.14 ± 5.49 | 263.54 ± 4.48 | 208.10 ± 4.36 | 165.90 ± 3.30 |
| 36 | 151.95 ± 2.88 | 146.16 ± 2.92 | 127.22 ± 2.16 | 93.67 ± 1.59 |
| ΣCou | 1153.35 | 1081.16 | 954.09 | 638.95 |

[a] Extraction type: USE – ultrasound extraction (50°C), MWAE – microwave-assisted extraction (20°C), BWBE – boiled water bath extraction (95°C), RTE – room temperature extraction (20°C). [b] Compounds: **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid. Tr. – traces. Extraction conditions: 400 mg of plant material was extracted by 10 mL of water-methanol (20:80, *v*/*v*) for 30 min and after centrifuging and filtering was analyzed in mc-HPC-UV.

**Table S10.** Content of compounds **9**, **11**, **12**, **15**, **17**, **27**, **31**, **i-xi** and sum of coumarins (ΣCou) in roots of 17 *Phlojodicarpus* samples, mg/g of dry plant weight.

| Compd [a] | *P. sibiricus* (S) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SY1 | SY2 | SY3 | SB1 | SB2 | SB3 | SB4 | SM1 | SM2 | SC1 | SC2 | SC3 |
| **9** | 2.33 | 2.52 | 2.07 | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. |
| **11** | 3.94 | 4.70 | 3.32 | Tr. | Tr. | Tr. | Tr. | 1.42 | 1.25 | 20.62 | 21.52 | 19.69 |
| **12** | 0.71 | 1.49 | 0.21 | 1.71 | 0.94 | 1.28 | 2.06 | Tr. | Tr. | 0.72 | 0.54 | 0.95 |
| **15** | 67.08 | 80.14 | 73.14 | 0.40 | 0.42 | 0.59 | 0.22 | 1.90 | 1.27 | 3.37 | 4.49 | 3.17 |
| **17** | 0.39 | 0.53 | 0.94 | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. | Tr. |
| **27** | 5.82 | 7.47 | 7.04 | 6.25 | 7.16 | 6.33 | 5.11 | 7.63 | 8.11 | 2.78 | 2.04 | 2.11 |
| **31** | 1.55 | 1.39 | 1.30 | 1.59 | 1.63 | 1.50 | 1.11 | 1.53 | 0.97 | 0.41 | 0.35 | 0.30 |
| **i** | Tr. | Tr. | Tr. | 0.32 | 0.22 | 0.12 | 0.35 | 0.08 | 0.02 | Tr. | Tr. | Tr. |
| **ii** | Tr. | Tr. | Tr. | 4.17 | 4.27 | 5.16 | 2.39 | 3.39 | 4.28 | 0.75 | 1.27 | 1.30 |
| **iii** | Tr. | Tr. | Tr. | 4.79 | 5.83 | 7.59 | 2.10 | 2.95 | 3.16 | 0.42 | 0.12 | 0.10 |
| **iv** | Tr. | Tr. | Tr. | 1.20 | 0.33 | 0.57 | 0.52 | 0.43 | 0.51 | Tr. | Tr. | Tr. |
| **v** | Tr. | Tr. | Tr. | 1.56 | 0.37 | 1.27 | 1.09 | Tr. | Tr. | Tr. | Tr. | Tr. |
| **vi** | Tr. | Tr. | Tr. | 3.35 | 3.94 | 4.12 | 4.57 | 2.12 | 3.57 | 0.22 | 0.26 | 0.41 |
| **vii** | Tr. | Tr. | Tr. | 8.39 | 9.37 | 8.15 | 8.73 | 1.82 | 1.63 | 0.28 | 0.04 | 0.35 |
| **viii** | Tr. | Tr. | Tr. | 7.11 | 7.00 | 5.25 | 5.14 | 2.65 | 2.84 | Tr. | Tr. | Tr. |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ix** | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. |
| **x** | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. |
| **xi** | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. | N.d. |
| ΣCou | 81.82 | 98.24 | 88.02 | 40.84 | 41.48 | 41.93 | 33.39 | 25.92 | 27.61 | 29.57 | 30.63 | 28.38 |

| Compd [a] | *P. villosus* (V) | | *P. turczaninovii* (T) | | |
|---|---|---|---|---|---|
| | **VY1** | **VB1** | **TT1** | **TT2** | **TB1** |
| **9** | Tr. | Tr. | 0.39 | 0.45 | 0.94 |
| **11** | 7.63 | 8.24 | 0.34 | 0.32 | 0.14 |
| **12** | 2.46 | 2.06 | N.d. | N.d. | N.d. |
| **15** | 2.12 | 1.37 | 6.09 | 6.37 | 8.22 |
| **17** | Tr. | Tr. | N.d. | N.d. | N.d. |
| **27** | 17.71 | 21.15 | 3.90 | 4.63 | 3.12 |
| **31** | 1.74 | 1.59 | 1.45 | 0.32 | 1.67 |
| **i** | Tr. | Tr. | Tr. | Tr. | Tr. |
| **ii** | 0.62 | 0.34 | Tr. | Tr. | Tr. |
| **iii** | Tr. | Tr. | 1.24 | 0.87 | 1.10 |
| **iv** | 1.62 | 1.73 | 1.27 | 1.20 | 0.63 |
| **v** | 1.28 | 0.94 | 1.09 | 1.63 | 1.50 |
| **vi** | 2.62 | 3.15 | 0.53 | 0.28 | 0.11 |
| **vii** | 12.22 | 14.67 | 0.39 | 0.26 | 0.51 |
| **viii** | 2.73 | 3.75 | Tr. | Tr. | Tr. |
| **ix** | N.d. | N.d. | 23.90 | 25.63 | 30.47 |
| **x** | N.d. | N.d. | 24.03 | 21.05 | 32.15 |
| **xi** | N.d. | N.d. | 12.80 | 14.89 | 12.07 |
| ΣCou | 52.75 | 58.99 | 77.42 | 44.90 | 92.63 |

[a] Compounds: **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid. Tr. – traces. N.d. – not detected. Compounds numbered as **i**–**xi** – unidentified compounds.

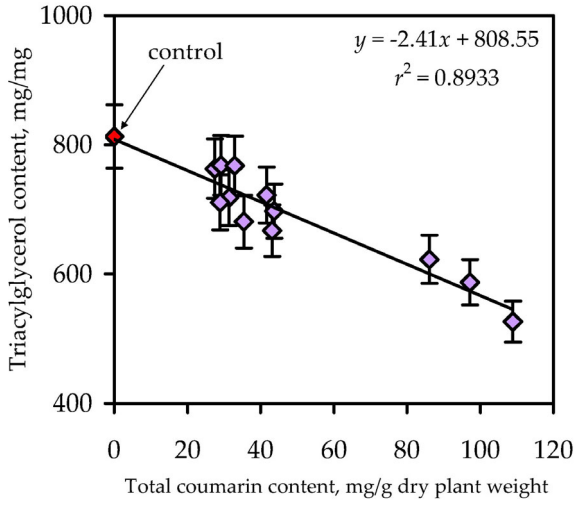
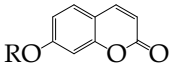



**Figure S1.** Correlation graph between total coumarin content (mg/g dry plant weight) in 12 samples of *Phlojodicarpus sibiricus* roots and their influence on tiacylglycerol (TG) content in 3T3-L1 adipocytes (μg/mg protein). The following data in Table used to build the graph.

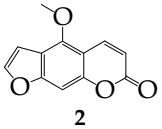
**Table for Figure S1.** Total coumarins content (TCC) in *P. sibiricus* roots and influence of *Phlojodicarpus* extracts on tiacylglycerol (TG) content in 3T3-L1 adipocytes [1].

| Sample no | TCC, mg/g [a,2] | TG, μg/mg protein [b,3] |
|---|---|---|
| SY1 (50 μg/mL) | 86.14 ± 1.72 | 622.7 ± 24.9 * |
| SY2 (50 μg/mL) | 108.94 ± 1.96 | 526.4 ± 20.5 * |
| SY3 (50 μg/mL) | 97.20 ± 1.74 | 587.3 ± 19.9 * |
| SB1 (50 μg/mL) | 41.63 ± 0.71 | 722.1 ± 28.8 * |
| SB2 (50 μg/mL) | 43.15 ± 0.69 | 667.2 ± 22.6 * |
| SB3 (50 μg/mL) | 43.76 ± 0.92 | 697.4 ± 23.6 * |
| SB4 (50 μg/mL) | 35.37 ± 0.64 | 681.4 ± 29.2 * |
| SM1 (50 μg/mL) | 27.54 ± 0.55 | 763.1 ± 31.2 |
| SM2 (50 μg/mL) | 29.18 ± 0.59 | 768.2 ± 30.7 |
| SC1 (50 μg/mL) | 31.40 ± 0.56 | 718.4 ± 25.8 * |
| SC2 (50 μg/mL) | 32.89 ± 0.51 | 767.6 ± 29.9 |
| SC3 (50 μg/mL) | 28.96 ± 0.43 | 711.0 ± 22.7 * |
| Control | - | 812.8 ± 25.1 |
| 5CQA (10 μg/mL) | - | 286.2 ± 11.45 * |

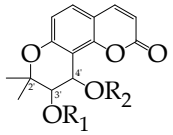
[1] Averages ± standard deviation were obtained from three ([a]) or four ([b]) different experiments. [2] Dry extract weight. [3] Values with asterisk (*) indicate statistically significant differences with the control groups at $p < 0.05$ by one-way ANOVA.



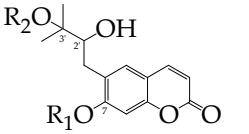
**1**: R = H
**27**: R = β-D-Api*f*-(1→6)-β-D-Glc*p*



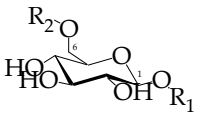
**2**



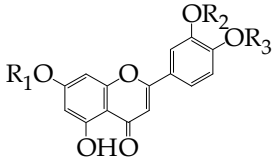
**3**: $R_1 = R_2 = H$
**4**: $R_1 = H$; $R_2 = CH_3$
**6**: $R_1 = H$; $R_2$ = Ac
**9**: $R_1 = R_2$ = Ac
**11**: $R_1 = H$; $R_2$ = Ang
**12**: $R_1$ = Ac; $R_2$ = i-Bu
**13**: $R_1$ = MeBu; $R_2$ = Ac
**14**: $R_1$ = Ac; $R_2$ = Ang
**15**: $R_1$ = i-Val; $R_2$ = Ac
**16**: $R_1$ = Ac; $R_2$ = i-Val
**17**: $R_1$ = Ac; $R_2$ = MeBu
**18**: $R_1 = R_2$ = Sen
**19**: $R_1 = R_2$ = Ang
**31**: $R_1$ = β-D-Glc*p*; $R_2 = H$



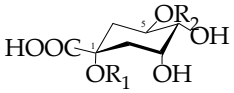
**28**: $R_1$ = β-D-Glc*p*; $R_2 = H$
**32**: $R_1 = H$; $R_2$ = β-D-Glc*p*



**33**: $R_1$ = Caf; $R_2 = H$
**35**: $R_1 = H$; $R_2$ = Caf



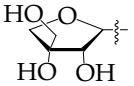
**37**: $R_1$ = β-D-Glc*p*; $R_2 = H$, $R_3 = CH_3$
**38**: $R_1$ = β-D-Glc*p*; $R_2 = CH_3$, $R_3 = H$



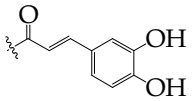
**34**: $R_1$ = Caf; $R_2 = H$
**36**: $R_1 = H$; $R_2$ = Caf

Ac
(acetyl)

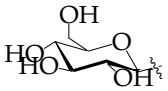
Ang
(angeloyl)



Api*f*
(apiofuranosyl)



Caf
(caffeoyl)



Glc*p*
(glucopyranosyl)

| i-Bu | i-Val | MeBu | Sen |
|---|---|---|---|
| (isobutyroyl) | (isovaleroyl) | (2-methylbutyroyl) | (senecioyl) |

**Figure S2.** Structures of reference standards used in present work. Abbreviations structures showed below.

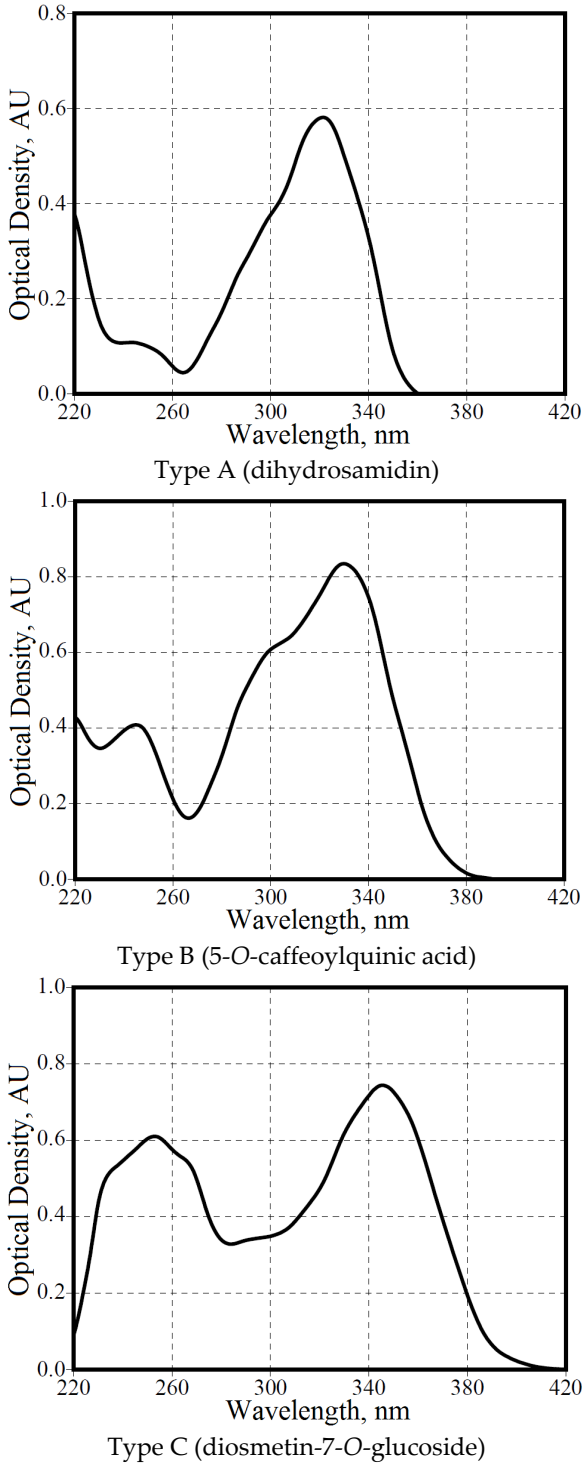



**Figure S3.** Types of UV spectral patterns.

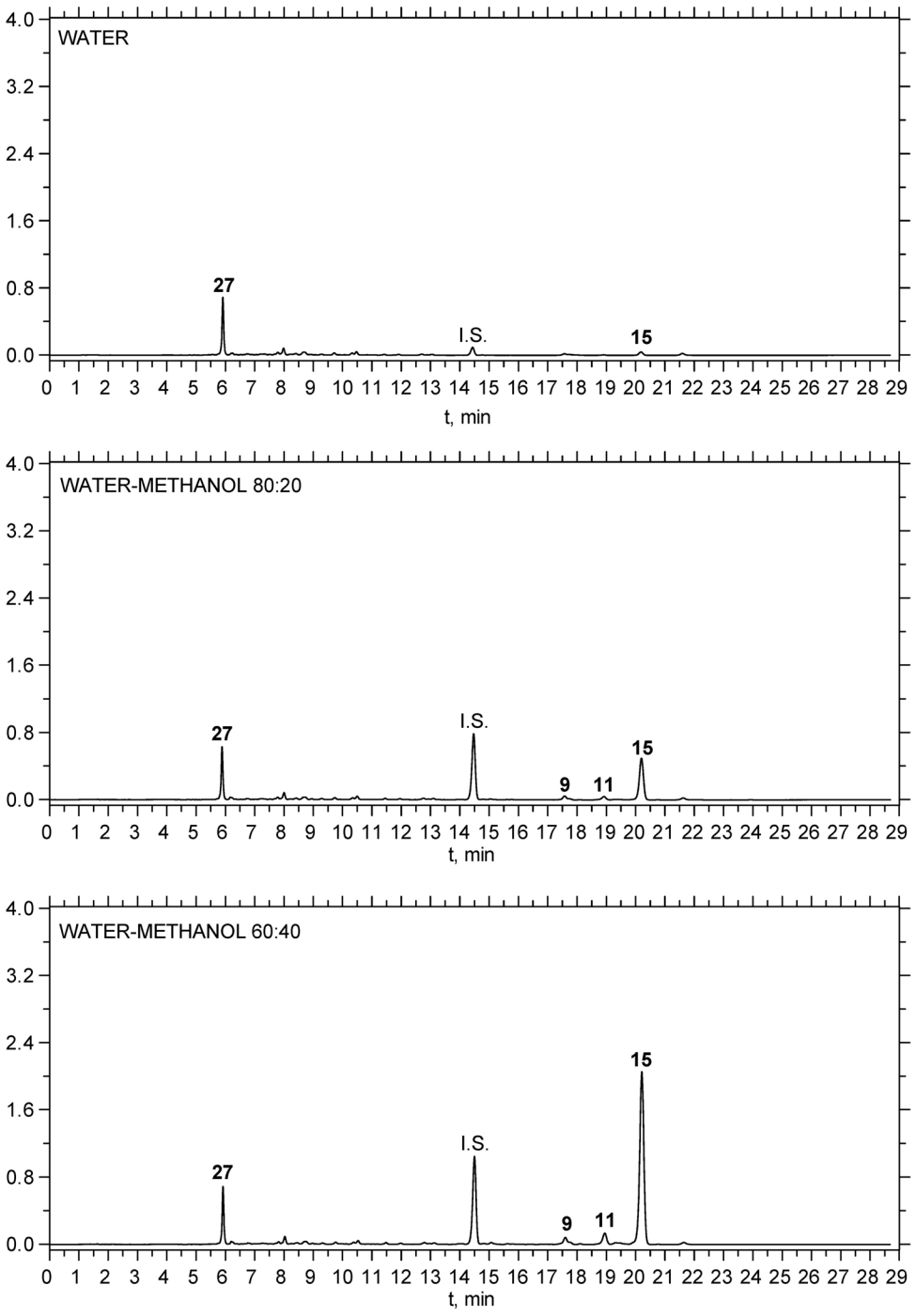

WATER
27
I.S.
15
WATER-METHANOL 80:20
27
I.S.
9
11
15
WATER-METHANOL 60:40
27
I.S.
9
11
15
t, min

**Figure S4**. *Cont*.

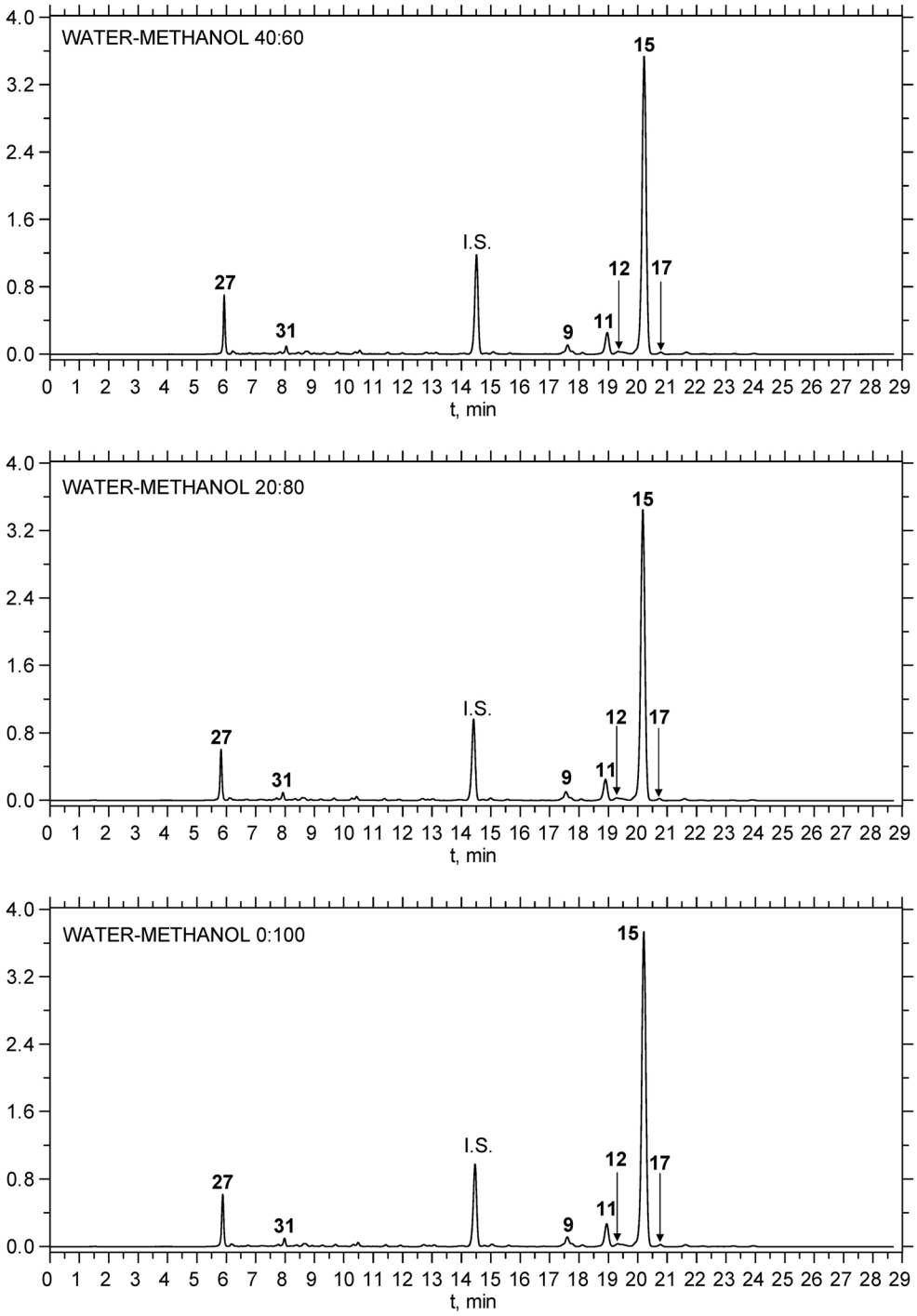



**Figure S4**. mcHPLC-UV chromatograms of *P. sibiricus* roots extracts prepared with various solvents. Compounds numbered as **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II). I.S. – internal standard (pimpinellin).

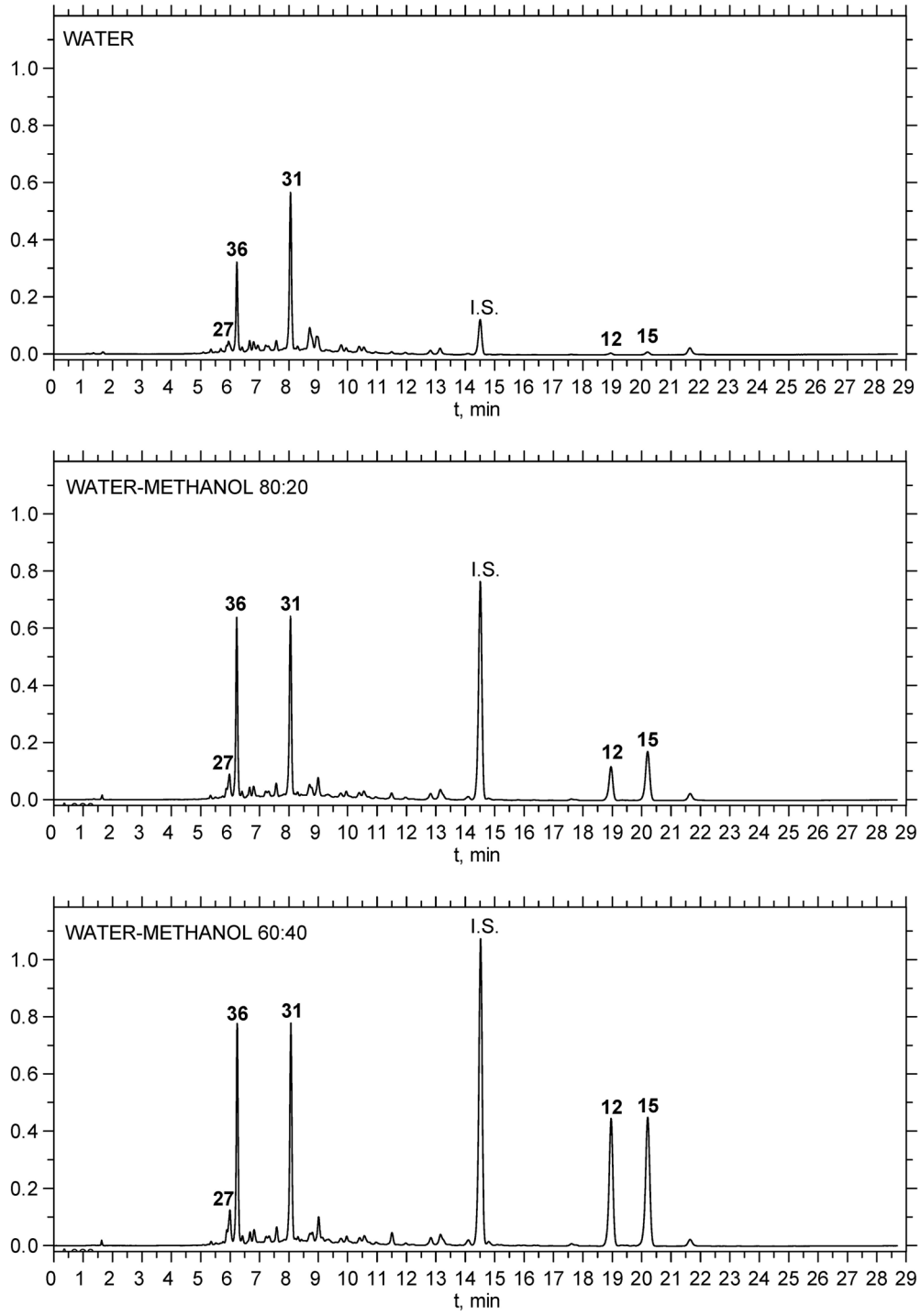

WATER
1.0
0.8
0.6
0.4
0.2
0.0
31
36
I.S.
27
12
15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
t, min
WATER-METHANOL 80:20
1.0
0.8
0.6
0.4
0.2
0.0
I.S.
36
31
27
12
15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
t, min
WATER-METHANOL 60:40
1.0
0.8
0.6
0.4
0.2
0.0
I.S.
36
31
12
15
27
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
t, min

**Figure S5**. *Cont*.

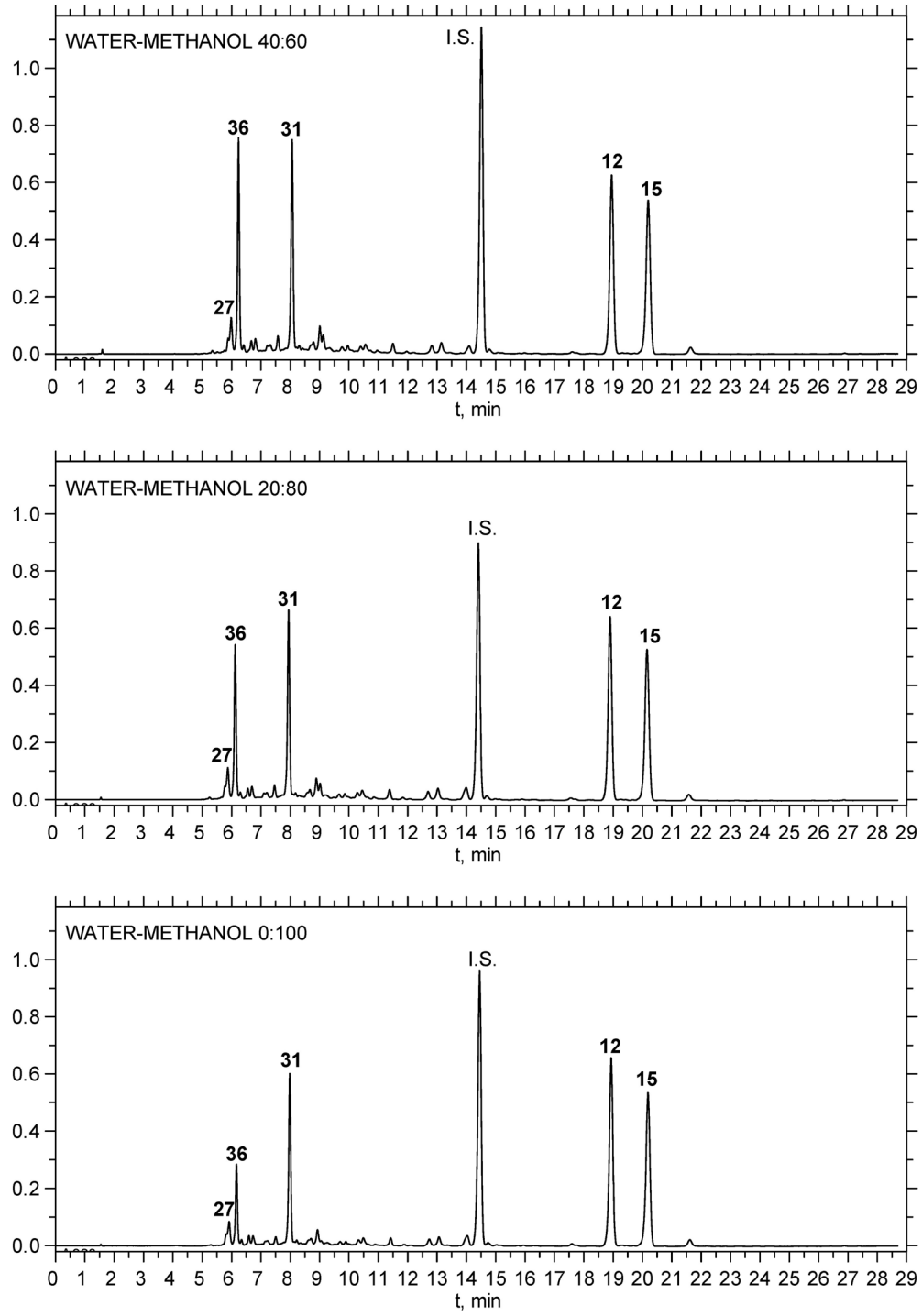



**Figure S5**. mcHPLC-UV chromatograms of *P. sibiricus* herb extracts prepared with various solvents. Compounds numbered as **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II), **36** – 5-*O*-caffeoylquinic acid. I.S. – internal standard (pimpinellin).

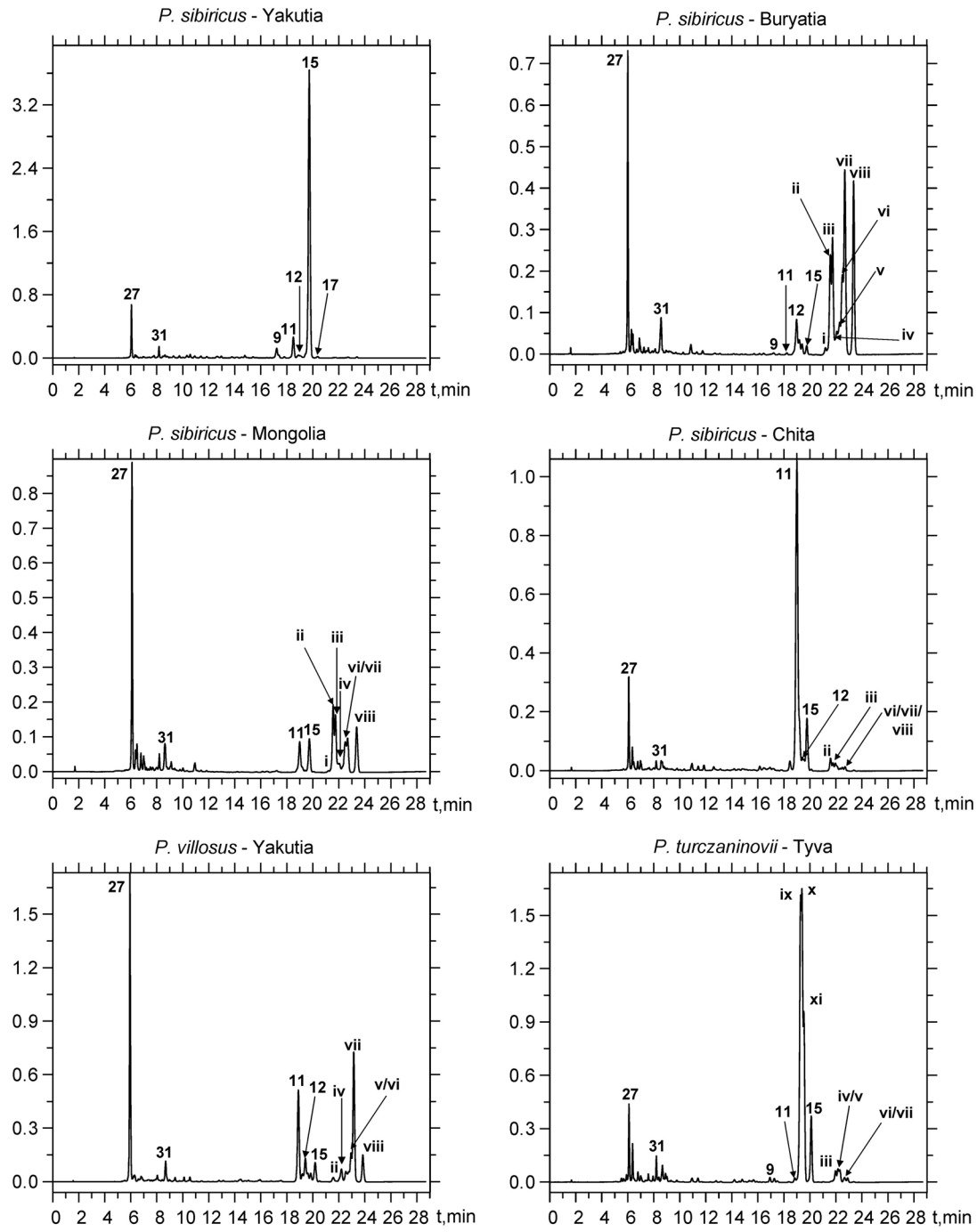



**Figure S6**. mc-HPLC-UV chromatograms of *Phlojodicarpus* root extracts from various regions. Compounds numbered as **9** – khellactone-3′,4′-di-*O*-acetyl ester, **11** – khellactone 4′-*O*-angeloyl ester (d-laserpitin), **12** – khellactone 3′-*O*-acetyl-4′-*O*-isobutyroyl ester (hyuganin D), **15** – khellactone 3′-*O*-isovaleroyl-4′-*O*-acetyl ester (dihydrosamidin), **17** – khellactone 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl) ester (hyuganin C), **27** – umbelliferone-7-*O*-(6′-apiosyl)-glucoside (6′-apiosylskimmin), **31** – khellactone-3′-*O*-glucoside (praeroside II). Compounds numbered as **i–xi** – unidentified compounds.