Supplementary file 1

Antidiabetic effect of *Saurauia bracteosa* through GSK-3β inhibition: Insights from phytochemical analysis, molecular docking, and glucose uptake in L6 cells

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S1. LC-MS/MS analysis

S. bracteosa extract (1 mg) was dissolved in 10 µL of methanol. One microliter of the sample was injected into the LC-MS/MS system at a flow rate of 0.2 mL/min. Chromatographic separation was carried out on an ACQUITY UPLC® BEH C18 column (Waters Corporation, Milford, Massachusetts, United States of America; pore size: 130 Å; particle size: 1.7 µm; internal diameter: 2.1 mm; length: 50 mm). The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) under reversed-phase conditions. The analysis was performed using a Waters ACQUITY UPLC I-Class system coupled with an Xevo G2-XS QTOF mass spectrometer (Waters Corporation, Manchester, United Kingdom). Mass spectra were acquired in positive electrospray ionization (ESI+) mode, scanning from m/z 100 to 1200.

S2. GC-MS analysis

Extract of *S. bracteosa* was identified using a gas chromatography-mass spectrometry (GC-MS) QP2010 SE system. under the following conditions: the column oven temperature was set at 80 °C, and the injection temperature was maintained at 300 °C. The analysis was conducted in split injection mode, with pressure-controlled flow at 42.3 kPa, a total flow rate of 117.5 mL/min, and a column flow rate of 0.74 mL/min. The linear velocity was set at 31.8 cm/sec, purge flow at 3.0 mL/min, and the split ratio was 153:1. The ion source temperature was 250 °C, and the interface temperature was 300 °C. Mass spectrometry was performed for 29 minutes, with a mass scan range of *m/z* 50.00 to 600.00.

The column used was an Rtx-5MS (film thickness: 0.25 µm; length: 30.0 m; inner diameter: 0.25 mm; maximum temperature: 330 °C). Method validation was conducted through linearity assessment, which included linear calibration analysis and determination of the correlation coefficient (R^2) from the calibration curve.

GC-MS data were acquired and processed using GCMSsolution Workstation Software (Shimadzu Corporation, Kyoto, Japan). Data analysis—including calibration, quantification of unknown compounds, and report generation—was also carried out using this software. The identified compounds showed similarity scores exceeding 90% when matched with entries in the reference database.

S3. Specific instrument settings for UPLC–MS analysis of ursolic acid and quercetin

Parameter	**Ursolic acid**	**Quercetin**
Instrument	ACQUITY UPLC H-Class system with PDA detector and Xevo™ TQ-D triple-quadrupole MS (Waters Corp., USA)	Same
Column	ACQUITY BEH C18 (2.1 × 100 mm, 1.7 µm)	Same
Column temperature	40 °C	40 °C
Mobile phase	Water : acetonitrile (30 : 70, v/v)	(A) Water + 0.1 % formic acid; (B) Acetonitrile + 0.1 % formic acid
Elution program	Isocratic	Gradient (10–100 % B)
Flow rate	0.3 mL/min	0.3 mL/min
Injection volume	3 µL	3 µL
Detection wavelength (PDA)	196 nm	208 nm
MS mode	ESI (negative ionization)	ESI (negative ionization)
Capillary voltage	2.5 kV	2.5 kV
Cone voltage	65 V	40 V
Collision energy	35 V	20 V
Validation range (linearity)	7.8125–250 ppm	0.24–7.84 ppm
Wavelength for calibration	220 nm	360 nm
LOD / LOQ	3.125 ppm / calculated from SD/α (n = 7)	0.008 ppm / calculated from SD/α (n = 7)
Precision (RSD)	≤ 15 % (intra-/inter-day)	≤ 15 % (intra-/inter-day)

Parameter	Ursolic acid	Quercetin
Sample preparation	10 mg extract → 1,000 ppm stock → analyzed under identical conditions	Same

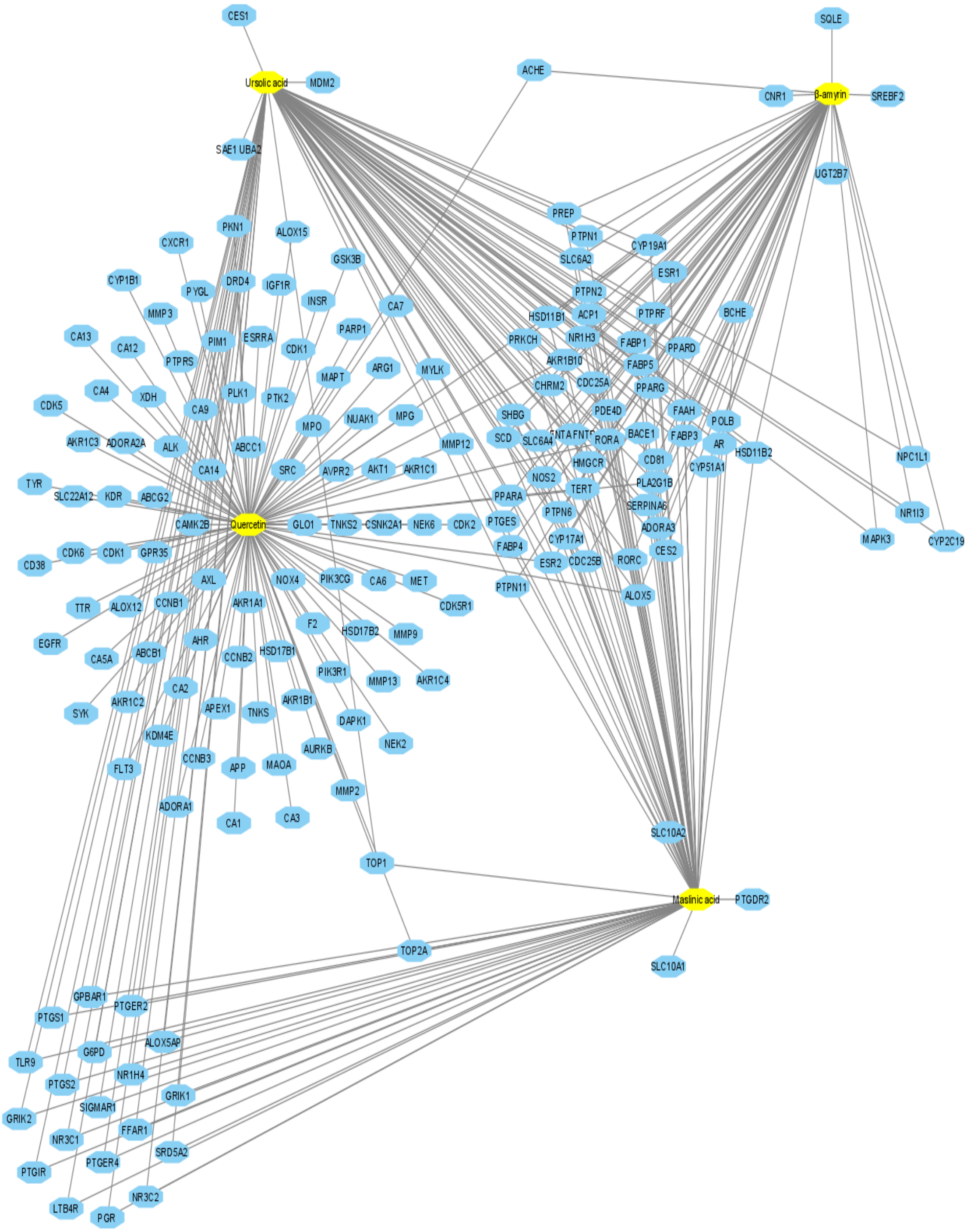
S4. Secondary metabolites identified in *Saurauia bracteosa* leaf extract by gas chromatography-mass spectrometry (GC-MS)

No	Compound name/library ID	Molecular formula	Retention time (minutes)	Area (%)	Quality (%)
1	Benzene, 1-(1,5-dimethyl-4-hexenyl) -4-methyl	$C_{15}H_{22}$	14.6	0.6	99
2	(R)-1-methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	$C_{15}H_{24}$	14.9	0.3	96
3	2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	$C_{11}H_{16}O_2$	15.3	0.3	98
4	6-hydroxy-4,4,7a-trimethyl-5,6,7,7 a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	18.0	1.2	99
5	Neophytadiene	$C_{20}H_{38}$	18.5	1.0	99
6	3-methylene-7,11-dimethyl-1-dodecene	$C_{15}H_{28}$	18.7	0.2	91
7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	19.4	3.9	99
8	N-hexadecanoic acid	$C_{16}H_{32}O_2$	19.8	3.3	99
9	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	20.4	0.4	96
10	9,12-octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	21.1	0.8	99
11	9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	21.1	2.4	99
12	Phytol	$C_{20}H_{40}O$	21.2	16.2	95
13	Methyl stearate	$C_{19}H_{38}O_2$	21.3	2.2	99
14	Z,Z-10,12-hexadecadien-1-ol acetat	$C_{19}H_{34}O_2$	21.5	1.8	91
15	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	21.7	0.3	95
16	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	23.1	0.5	91
17	4,8,12,16-tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	23.3	0.2	99
18	Pentacosane	$C_{25}H_{52}$	24.6	0.3	95
19	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	24.8	0.1	95
20	1-tetracosene	$C_{24}H_{48}$	26.0	4.1	95
21	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	26.3	0.2	99
22	Cyclooctacosane	$C_{28}H_{56}$	26.7	0.1	99
23	Squalene	$C_{30}H_{50}$	26.9	1.7	95
24	α-tocospiro A	$C_{30}H_{52}O_4$	27.3	1.8	99
25	1-hexacosene	$C_{26}H_{52}$	27.5	1.0	99
26	γ-tocopherol	$C_{28}H_{48}O_2$	28.8	0.3	92
27	Vitamin E	$C_{29}H_{50}O_2$	29.6	2.3	99
28	Ergost-5-en-3-ol, (3β)-	$C_{28}H_{48}O$	30.9	0.2	95
29	Stigmasterol	$C_{29}H_{48}O$	31.2	5.9	99

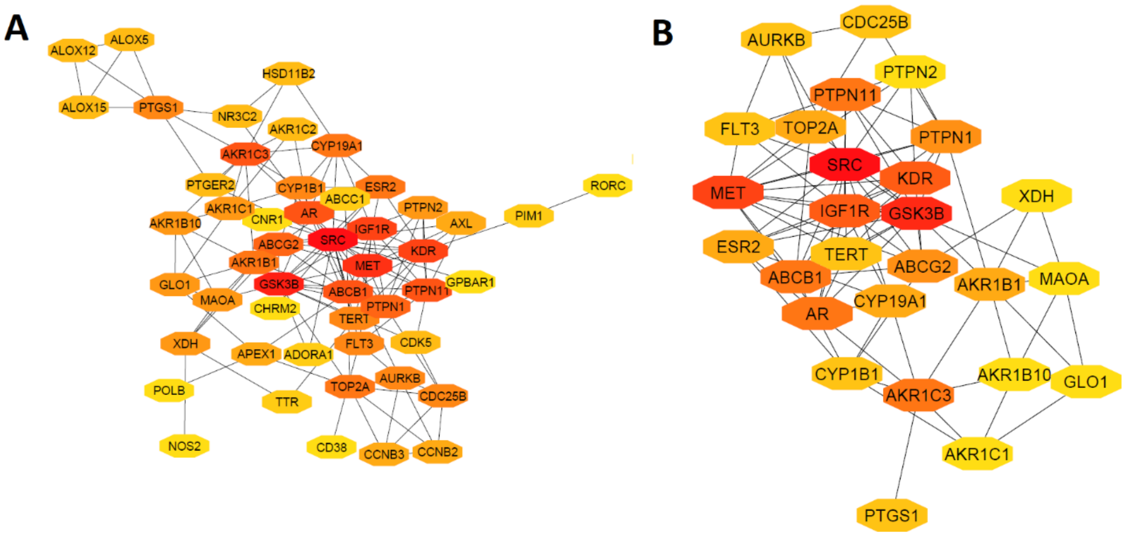
No	Compound name/library ID	Molecular formula	Retention time (minutes)	Area (%)	Quality (%)
30	γ-sitosterol	$C_{29}H_{50}O$	32.0	13.5	99
31	β-amyrone	$C_{30}H_{48}O$	32.2	1.5	97
32	β-amyrin	$C_{30}H_{50}O$	32.7	3.6	99
33	α-amyrin	$C_{30}H_{50}O$	33.5	12.4	99

Abbreviations: Library ID, library identification

S5. The predicted genes were identified using SwissTargetPrediction. Yellow nodes indicate compound genes and blue nodes indicate targeted genes.



S6. Protein–protein interaction network of 51 target genes linked to *Saurauia bracteosa* compounds. (A) Gene interaction network. (B) Twenty-seven hub proteins; color gradient (yellow–red) denotes increasing interaction degree.



Connected protein in protein-protein interaction

proto-oncogene tyrosine-protein kinase Src (SRC), glycogen synthase kinase-3 β (GSK3β), hepatocyte growth factor receptor (MET), vascular endothelial growth factor receptor (KDR), insulin-like growth factor 1 receptor (IGF1R), signaling lymphocytic activation molecule (PTPN11), aldo-keto reductase family 1 member C3 (AKR1C3), aflatoxin B1 aldehyde reductase member 3 (AR), ABC transporter B family member 1 (ABCB1), tyrosine-protein phosphatase non-receptor type 1 (PTPN1), ABC transporter G family member 2 (ABCG2), aromatase (CYP19A1), DNA topoisomerase 2-alpha (TOP2A), estrogen receptor beta (ESR2), aldo-keto reductase family 1 member B1 (AKR1B1), prostaglandin G/H synthase 1 (PTGS1), cytochrome P450 1B1 (CYP1B1), telomerase reverse transcriptase (TERT), aurora kinase B (AURKB), receptor-type tyrosine-protein kinase FLT3 (FLT3), M-phase inducer phosphatase 2 (CDC25B), glycolate oxidase 1 (GLO1), aldo-keto reductase family 1 member B10 (AKR1B10), aldo-keto reductase family 1 member C1 (AKR1C1), xanthine dehydrogenase (XDH), tyrosine-protein phosphatase non-receptor type 2 (PTPN2), and amine oxidase (MAOA).

S7. Molecular docking of *Saurauia bracteosa* compounds with GSK3β

Secondary metabolites	*Docking score* 1 (kcal/mol)	*Docking score* 2 (kcal/mol)	*Docking score* 3 (kcal/mol)	Average of ∑ *docking score* (kcal/mol)
Native ligand (Z)-1h,1'h [2,3']-Biindolylidene-3,2'-Dione-3-Oxime	-9.9	-9.8	-9.9	-9.8
Pioglitazone	-7.5	-6.9	-7.1	-7.1

Secondary metabolites	*Docking score* 1 (kcal/mol)	*Docking score* 2 (kcal/mol)	*Docking score* 3 (kcal/mol)	Average of ∑ *docking score* (kcal/mol)
Metformin	-5.0	-4.8	-5.0	-4.9
Quercetin	-9.1	-9.1	-9.1	-9.1
Ursolic acid	-8.1	-8.1	-8.1	-8.1
Maslinic acid	-7.8	-7.6	-7.6	-7.6
β – amyrin	-7.5	-7.4	-7.4	-7.4

S8. 2D visualization of the interacting amino acid residues with the GSK3β receptor. (A) native ligand (Z)-1h,1'h [2,3']-Biindolylidene-3,2'-Dione-3-Oxime; (B) metformin; (C) pioglitazone; (D) quercetin; (E) ursolic acid; (F) β-amyrin; and (G) maslinic acid.