Electronic Supplementary Material (ESI) for Food & Function.

S. Table 1. Isoflavones analyzed from three legume ethanol extracts by using UHPLC-DAD-ESI-Orbitrap HRMS

Compound	Black soybean 70% ethanol extract	Soybean 70% ethanol extract	Adzuki bean 50% ethanol extract
Daidzein	**+**	**+**	**+**
Genistein	**+**	**+**	**+**
Glycitein	**+**	**+**	**ND**
Daidzin	**+**	**+**	**+**
Genistin	**+**	**+**	**+**
Glycitin	**+**	**+**	**ND**
6’’-O-Malonyldaidzin	**+**	**+**	**+**
6’’-O-Malonlgenistin	**+**	**+**	**ND**
6’’-O-Malonylglycitin	**+**	**+**	**ND**
6’’-O-Acetyldaidzin	**+**	**+**	**+**
6’’-O-Acetylgenistin	**+**	**+**	**ND**
6’’-O-Acetylglycitin	**+**	**+**	**ND**
Quercetin	**+**	**ND**	**+**

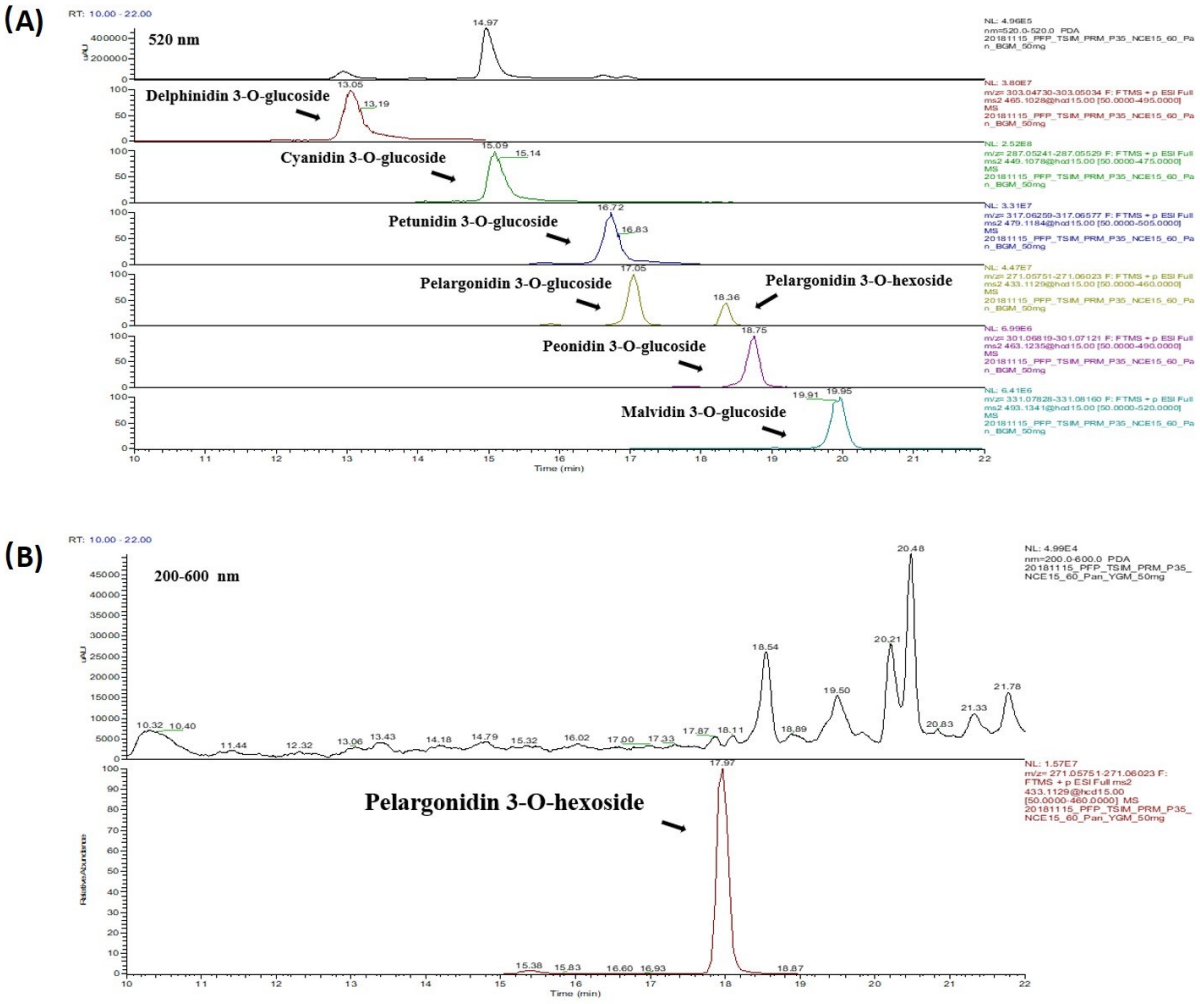
Rutin	**+**	**+**	**+**

Symbol (+) indicates the presence of isoflavones in the extract while ND stands for not detected.

S. Table 2. Anthocyanins analyzed from three legume ethanol extracts by using UHPLC-DAD-ESI-Orbitrap HRMS.

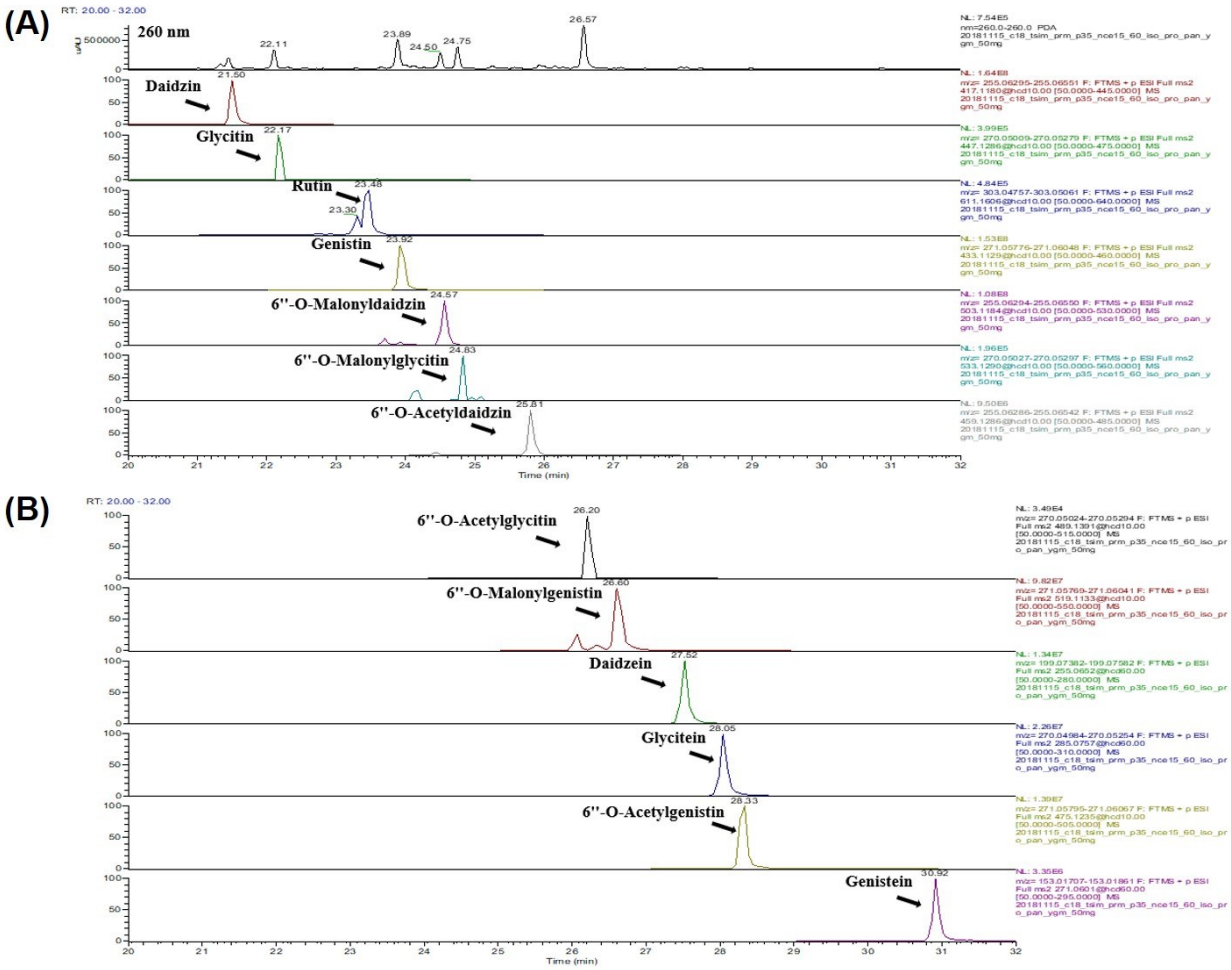
Compound	**Black soybean** **70% ethanol extract**	**Soybean** **70% ethanol extract**	**Adzuki bean** **50% ethanol extract**
Delphinidin 3-O-glucoside	**+**	**ND**	**ND**
Cyanidin 3-O-glucoside	**+**	**ND**	**ND**
Petunidin 3-O-glucoside	**+**	**ND**	**ND**
Pelargonidin 3-O-glucoside	**+**	**ND**	**ND**
Pelargonidin 3-O-hexoside	**+**	**+**	**ND**
Peonidin 3-O-glucoside	**+**	**ND**	**ND**
Malvidin 3-O-glucoside	**+**	**ND**	**ND**

Symbol (+) indicates the presence of anthocyanins in the extract while ND stands for not detected.

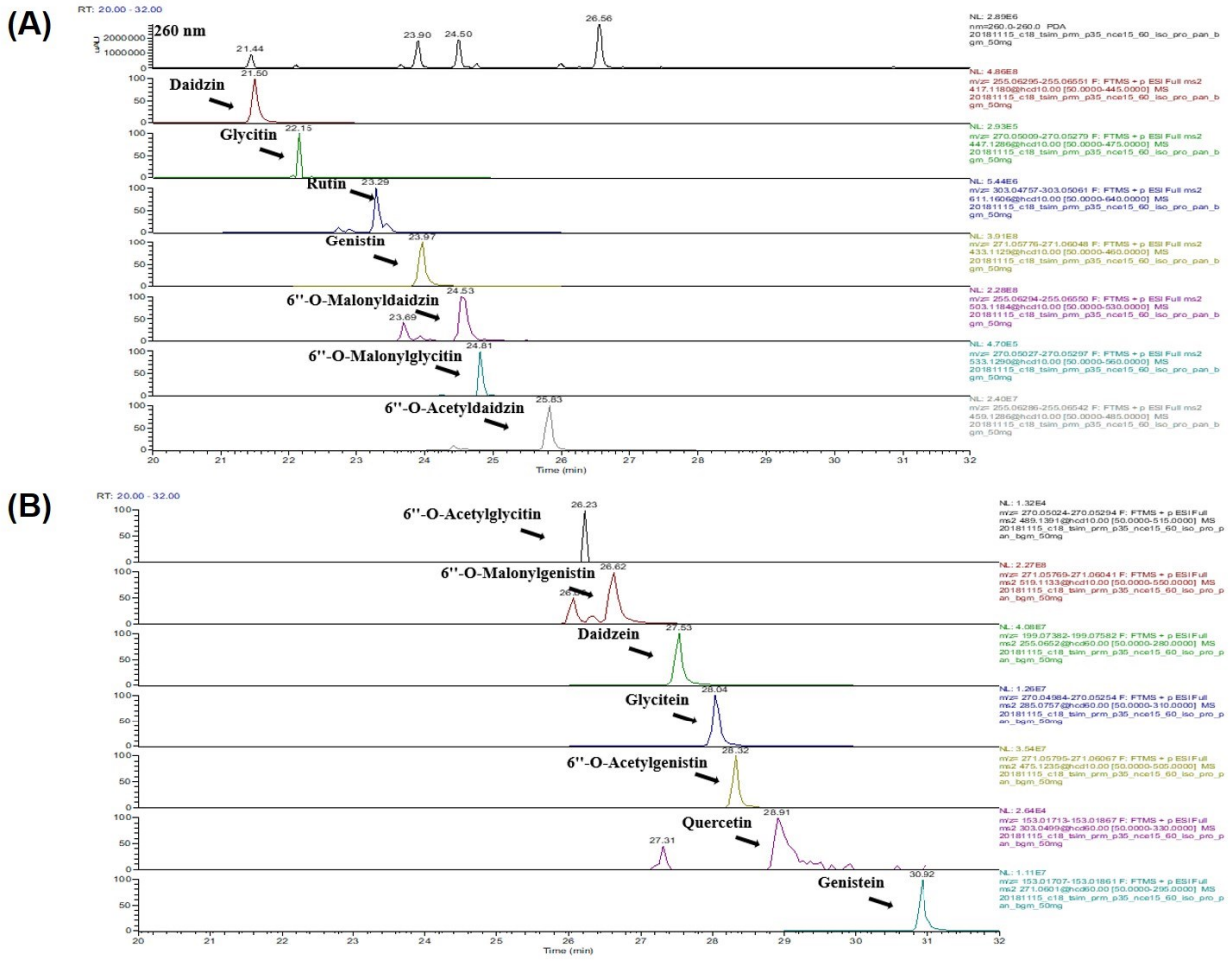


Supplemental Fig 1. The 520 nm absorption and product ion chromatogram of anthocyanins in Glycine max (L) merr sample (A) black soybean and (B) soybean. Chromatographic conditions: Column: Phenomenex Kinetex PFP column (150 mm × 2.1 mm, 2.6 μm); Mobile phase: 2% formic acid in deionized water / 2% formic acid in acetonitrile; Linear gradient elution: from 3% to 17% acetonitrile in 2% formic acid over 0-20 min; Flow rate: 0.2 mL/min; Column temperature: 50°C; Injection volume 5 μL; Time range: 10-22 min. Mass spectrometric conditions: Ion source: HESI; Source voltage +3.5 kV; Capillary temperature: 263°C; S-lens RF level: 55; Aux gas heater temp.: 425°C; Sheath Gas Flow Rate: 50 Arb; Aux Gas Flow Rate : 13 Arb; Sweep Gas Flow Rate: 0

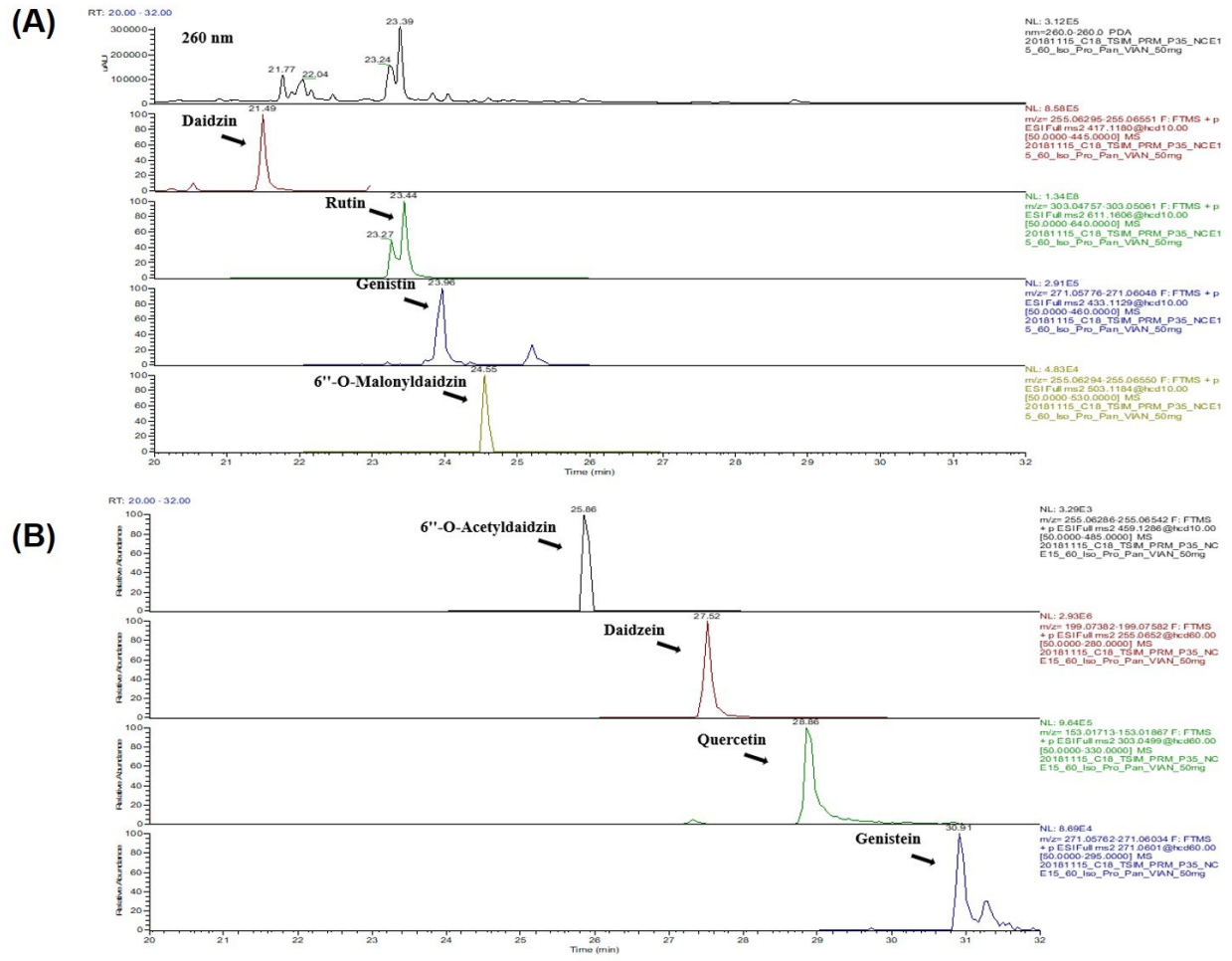
Arb. Q-Exactive Orbitrap HRMS: Resolution: 17500; AGC target: 2e5; Maximum injection time: 50 ms; Isolation window: 2.0 m/z; Normalized Collision Energy: 15%.



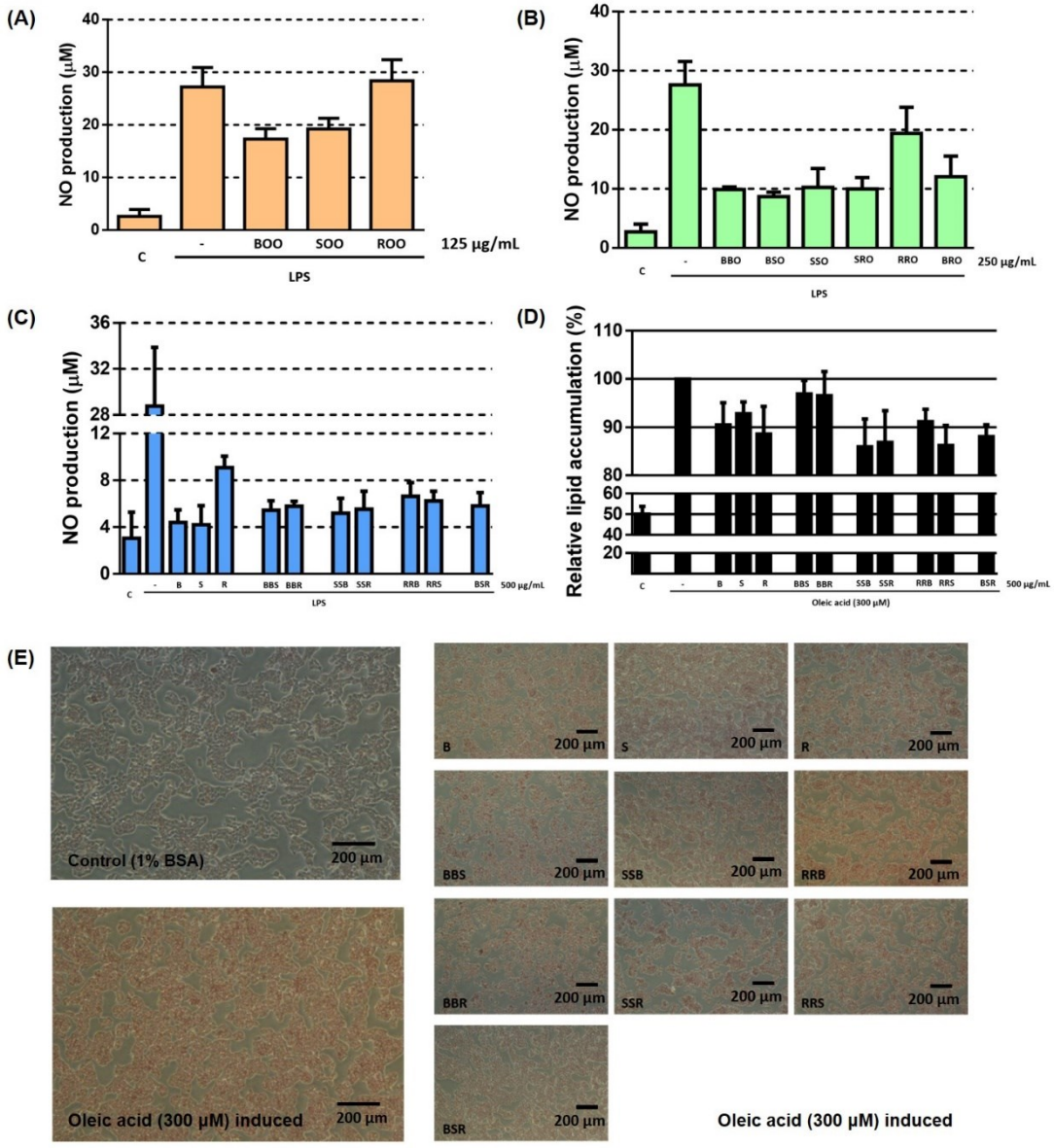
Supplemental Fig 2. The 260 nm absorption and product ion chromatogram of the flavonoids in Glycine max (L.) merr sample (soybean). Chromatographic conditions: Column: Phenomenex Kinetex C18 column (150 mm × 2.1 mm, 2.6 μm); Mobile phase: 0.1% formic acid in deionized water / 0.1% formic acid in acetonitrile; Linear gradient elution: Multistep; Flow rate: 0.3 mL/min; Column temperature: 25°C; Injection volume 5 μL; Time range: 20-32 min. Mass spectrometric conditions: Ion source: HESI; Source voltage +3.5 kV; Capillary temperature: 263°C; S-lens RF level: 55; Aux gas heater temp.: 425°C; Sheath Gas Flow Rate: 50 Arb; Aux Gas Flow Rate : 13 Arb; Sweep Gas Flow Rate: 0 Arb. Q-Exactive Orbitrap HRMS: Resolution: 17500; AGC target: 2e5; Maximum injection time: 50 ms; Isolation window: 2.0 m/z; Normalized Collision Energy:10%, 60%.



Supplemental Fig 3. The 260 nm absorption and product ion chromatogram of the flavonoids in Glycine max (L.) merr sample (Black soybean). Chromatographic conditions: Column: Phenomenex Kinetex C18 column (150 mm × 2.1 mm, 2.6 μm); Mobile phase: 0.1% formic acid in deionized water / 0.1% formic acid in acetonitrile; Linear gradient elution: Multistep; Flow rate: 0.3 mL/min; Column temperature: 25°C; Injection volume 5 μL; Time range: 20-32 min. Mass spectrometric conditions: Ion source: HESI; Source voltage +3.5 kV; Capillary temperature: 263°C; S-lens RF level: 55; Aux gas heater temp.: 425°C; Sheath Gas Flow Rate: 50 Arb; Aux Gas Flow Rate : 13 Arb; Sweep Gas Flow Rate: 0 Arb. Q-Exactive Orbitrap HRMS: Resolution: 17500; AGC target: 2e5; Maximum injection time: 50 ms; Isolation window: 2.0 m/z; Normalized Collision Energy: 10%, 60%.



Supplemental Fig 4. The 260 nm absorption and product ion chromatogram of the flavonoids in Vigna angularis sample (Adzuki bean). Chromatographic conditions: Column: Phenomenex Kinetex C18 column (150 mm × 2.1 mm, 2.6 μm); Mobile phase: 0.1% formic acid in deionized water / 0.1% formic acid in acetonitrile; Linear gradient elution: Multistep; Flow rate: 0.3 mL/min; Column temperature: 25°C; Injection volume 5 μL; Time range: 20-32 min. Mass spectrometric conditions: Ion source: HESI; Source voltage +3.5 kV; Capillary temperature: 263°C; S-lens RF level: 55; Aux gas heater temp.: 425°C; Sheath Gas Flow Rate: 50 Arb; Aux Gas Flow Rate : 13 Arb; Sweep Gas Flow Rate: 0 Arb. Q-Exactive Orbitrap HRMS: Resolution: 17500; AGC target: 2e5; Maximum injection time: 50 ms; Isolation window: 2.0 m/z; Normalized Collision Energy:10, 60%.



Supplemental Fig 5. The effect of selected legume ethanol extracts on different cell models. Anti-inflammatory effect of legume ethanol extracts on LPS (100 ng/mL)-induced murine macrophage 264.7 in final concentration of (A) 125 μg/mL, (B) 250 μg/mL and (C) 500 μg/mL. (D) Preventive effect of legume ethanol extracts on oleic acid (300 μM)-induced human liver cancer cell HepG2 in final concentration of 500 μg/mL.(E) Representative picture of (D) with oil red O staining. (under 100x magnification)

Supporting material

Cell culture and measurement of cell viability

Three cell lines, RAW 264.7 murine macrophage, 3T3-L1 preadipocyte and HepG2 human hepatoma cells were purchased from the American Type Culture Collection (Rockville, MD, USA). RAW264.7 and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS, GIBCO, Grand Island, NY, USA) while 3T3-L1 cells were cultured in DMEM supplemented with Fetal calf serum (FCS, GIBCO, Grand Island, NY, USA) in 37°C humidified atmosphere with 5% carbon dioxide. Both mediums were added with 100 mg/mL streptomycin and 100 U/mL penicillin. Cell viability of each cell line was determined by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-biphenultetrazolium bromide) assay to ensure the sample concentration exhibited no toxicity in the cell lines before further analysis.

NO measurement by nitrite assay

Nitrite assay is able to indicate the NO (nitrogen oxide) released from LPS-induced RAW264.7 cells, to be measured as nitrite concentration. After being cultured for 12 hours, RAW264.7 cells were treated with sample at 500 μg/mL and LPS (lipopolysaccharides) at the final concentration of 100 ng/mL for 24 hours. 100 μL of medium was added with same volume of Griess reagent to produce pinkish mixture. Diazo intermediate formed from reaction between nitrite and sulphanilamide from Griess reagent can further react with N-(1-Naphthyl-ethlenediamine dihydrochloride (NEDD) which results in pinkish azo compound. By reading the absorbance with enzyme-linked immune sorbent assay plate reader (ELISA reader, Dynatech MR-7000; Dynatech

Laboratories, Chantilly, VA, USA) at 550 nm, the nitrite production can be calculated with standard.

Lipid accumulation by oil red analysis

Measurement of lipid accumulation by using oil red in HepG2 and 3T3-L1 cells can be used to simulate triglyceride accumulation in liver and fat tissue. Briefly, HepG2 cells were seeded in 24-well plate at the density of 2×10^5 cells/mL and cultured for 12 hours. Sample and oleic acid were added at the final concentration of 500 μg/mL and 300 nM, respectively and the treatment took 24 hours. After incubation, cells were fixed with 4% formaldehyde in PBS solution for at least 3 hours in room temperature after being washed twice with PBS. Fixed cells were washed twice with PBS and incubated with oil red solution for 10 min at room temperature, followed by washing with PBS again. The cells were observed under microscope and the photographs were taken at 400× magnification. For quantification, oil red was extracted from stained lipid droplets by isopropanol and quantified by using ELISA reader at 550 nm. The result was presented as relative lipid content as compared to Oleic acid-induced group.

Similarly, 3T3-L1 murine preadipocytes were seeded at density of 2.5×10^4 cells/mL in DMEM supplemented with 10% FCS and cultured for 3 days. The original medium was changed into DMI (Differentiation medium I, aka DMEM (10% FBS) containing inducers including dexamethasone (DEX), rosiglitazone (Rosi), 3-Isobutyl-1-methylxanthine (IBMX) and insulin), along with intervention of samples at final concentration of 500 μg/mL. DMI was changed into DMII (Differentiation medium II, aka DMEM (10% FBS) containing insulin) every 2 days until day-8. At day 8, the cells were

washed and fixed and finally followed by oil red staining which the procedure is exactly same to the oil red staining of HepG2 as descripted above.

Isoflavones and anthocyanins analysis by using UHPLC-DAD-ESI-Orbitrap HRMS

Before analysis, 50-65 mg of legume extracts were weighed and followed by dissolving with 50% methanol solution (contained 2% formic acid) and fixed volume to 1 ml. Vortexed for 10s before 10 min sonification and filtrated with 0.22 μm Nylon filter. For the LC parameter, the column chosen was Kinetex PFP (2.1 mm ID x 150 mm, 2.6 μm) and the operation thermostat was set at 50ºC. Mobile phase A was composed of 2% formic acid in deionized water while mobile phase B was composed of 2% formic acid in acetonitrile. For the MS parameter, the HESI (Heated Electrospray Ionization) was performed at the sheath gas flow rate of 50 Arb; auxiliary gas unit flow rate at 13 Arb; the capillary temperature was set at 263ºC while auxiliary gas heater temperature was 425ºC, source voltage was 3500V+ and S-lens RF level was 55. Target-selected Ion monitoring and parallel reaction monitoring were employed for followed analysis. The former parameters were as following: orbitrap resolution of 35000, automatic gain control target value of 1×10^5 and maximum injection time of 100 ms while the latter acquisition was performed as following: orbitrap resolution of 17500, automatic gain control target value was 2×10^5 with the maximum injection time of 50 ms, and the individual isolation windows was 2 Th.