Guavinoside B from *Psidium guajava* alleviates acetaminophen-induced liver injury via regulating Nrf2 and JNK signaling pathways

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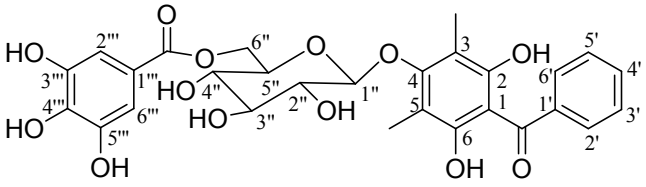
Isolation and identification of GUB

Guava fruit was sliced and dried under 40°C in an oven for 48 h, the yield dried product (6.0 kg) was then extracted with 70% ethanol (15 L × 3) at room temperature for 24 h. The supernatants were combined and evaporated under reduced pressure to afford crude ethanol extract (806.0 g). The residue was then suspended in water and partitioned successively with ethyl acetate (10 L × 5). After solvent removal, the ethyl acetate extract (89.4 g) was subjected to a MCI gel CHP-20P column, eluting with MeOH-H_2O system (0%, 20%, 40%, 60%, 80%, 100%) to afford six fractions A-F. Fraction E (11.0 g, 80% MeOH eluted fraction) was separated by silica gel column chromatography (100-200 mesh) with a gradient solvent system of CH_2Cl_2/CH_3OH (40:1 to 1:1) to obtain six subfractions E1-6. Subfraction E5 (5.0 g) was chromatographed on a Sephadex LH-20 column (MeOH as elution solvent), and then purified by an ODS column (MeOH/H_2O 60:40, v/v) to yield GUB 368.0 mg. The structure of GUB was identified by Mass, ^1H and ^{13}C NMR analysis，as well as the aid of comparison with literature report.[1]



GUB

^1H-NMR data (600 MHz, DMSO-d_6): δ 7.66 (d, 2H, J = 7.8 Hz, H-2′, 6′), 7.56 (t, 1H, J = 7.8 Hz, H-4′), 7.46 (t, 2H, J = 7.8 Hz, H-3′, 5′), 6,94 (s, 2H, H-2‴, 6‴), 4.61 (d, 1H, J = 7.8 Hz, H-1″), 4.37 (d, 1H, J = 12.0 Hz, H-6″a), 4.19 (dd, 1H, J = 12.0 Hz, 3.0 Hz, H-6″b), 3.28-3.43 (m, 4H, H-2″, 3″, 4″, 5″), 2.04 (s, 6H, -CH_3). ^{13}C-NMR (150 MHz,

DMSO-d_6): δ 197.5 (-C=O), 166.4 (-C=O), 156.4 (C-4), 153.0 (C-2, 6), 146.2 (C-3‴, 5‴), 139.4 (C-1′), 139.3 (C-4‴), 132.8 (C-4′), 129.3 (C-2′, 6′), 128.7 (C-3′, 5′), 119.8 (C-1‴), 113.6 (C-1), 111.2 (C-3, 5), 109.1 (C-2‴, 6‴), 104.6 (C-1″), 76.6 (C-3″), 74.7 (C-2″), 73.9 (C-5″), 69.8 (C-4″), 63.1 (C-6″), 10.4 (3, 5-CH_3).

Quantification of GUB by HPLC-UV method

Quantification of GUB in ethyl acetate extract of guava fruit was performed with HPLC-UV method on an Agilent 6420 Triple Quad mass spectrometer (Santa Clara, CA, USA), which was installed with an Agilent 1200 Infinity series high performance liquid chromatography (HPLC) system including an autosampler, quaternary pump and diode array detector (DAD). The ethyl acetate extract and GUB stock solution were prepared at the concentration of 10 mg/mL in MeOH, respectively. The stock solution of GUB was diluted with MeOH to a series of concentrations (0.015625 - 0.5 mg/mL) before using. YMC-Pack ODS column (150 × 4.6 mm, 5 μm) at the flow rate of 0.8 mL/min was used for quantification. The injection volume was 5 μL, and the detective wavelength was set at 280 nm. The gradient elution solvent system was consisting of solvent A (0.1% aqueous formic acid) and solvent B (MeOH) as following: 0 min, 30% B; 15min, 50% B; 15.1 min, 100% B; 20 min, 100% B; 20.1 min, 30% B. Between each injection, the balanced time was set for 5 min. Each analysis was conducted in triplicate. The GUB content was determined based on the established linear curve [$Y = 3863.4X + 2.8724$ ($R^2 = 0\ 9998$)]. The content of GUB in ethyl acetate extract was 0.4%, which was equivalent to 0.006% in dried guava fruits.

Reference:

1. K. Matsuzaki, R. Ishii, K. Kobiyama and S. Kitanaka, New benzophenone and quercetin galloyl glycosides from *Psidium guajava* L, *J. Nat. Med.*, 2010, **64**, 252-256.

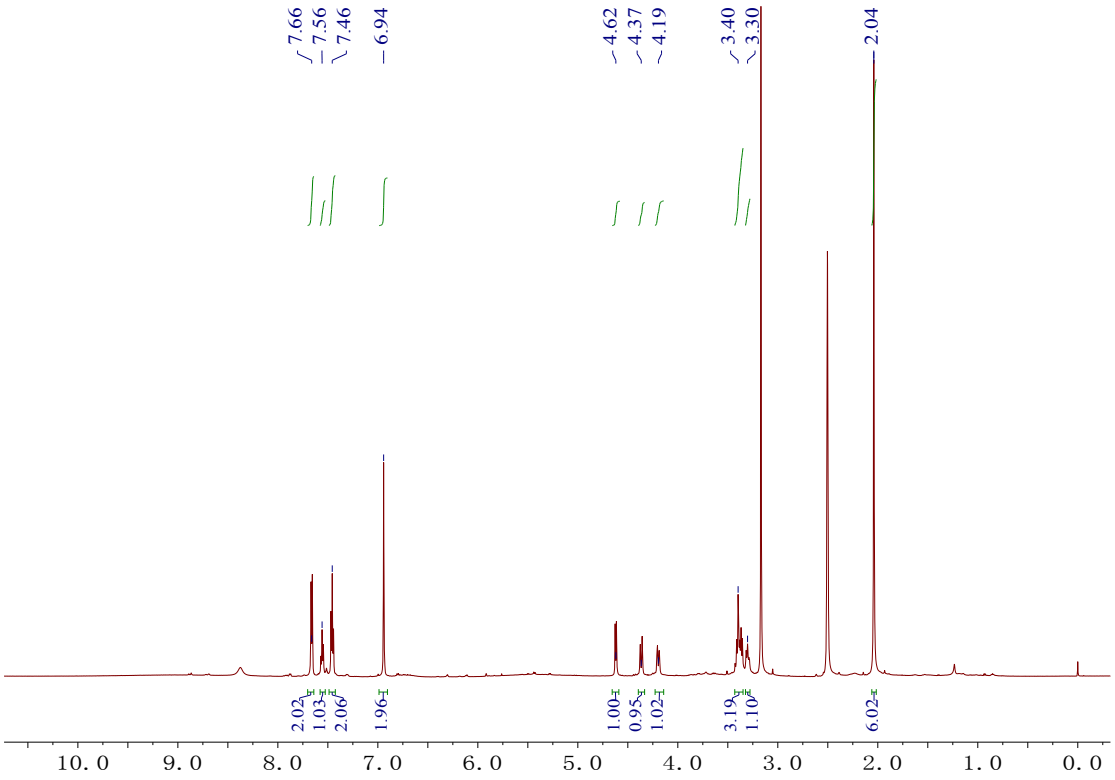


Figure S1. ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of GUB

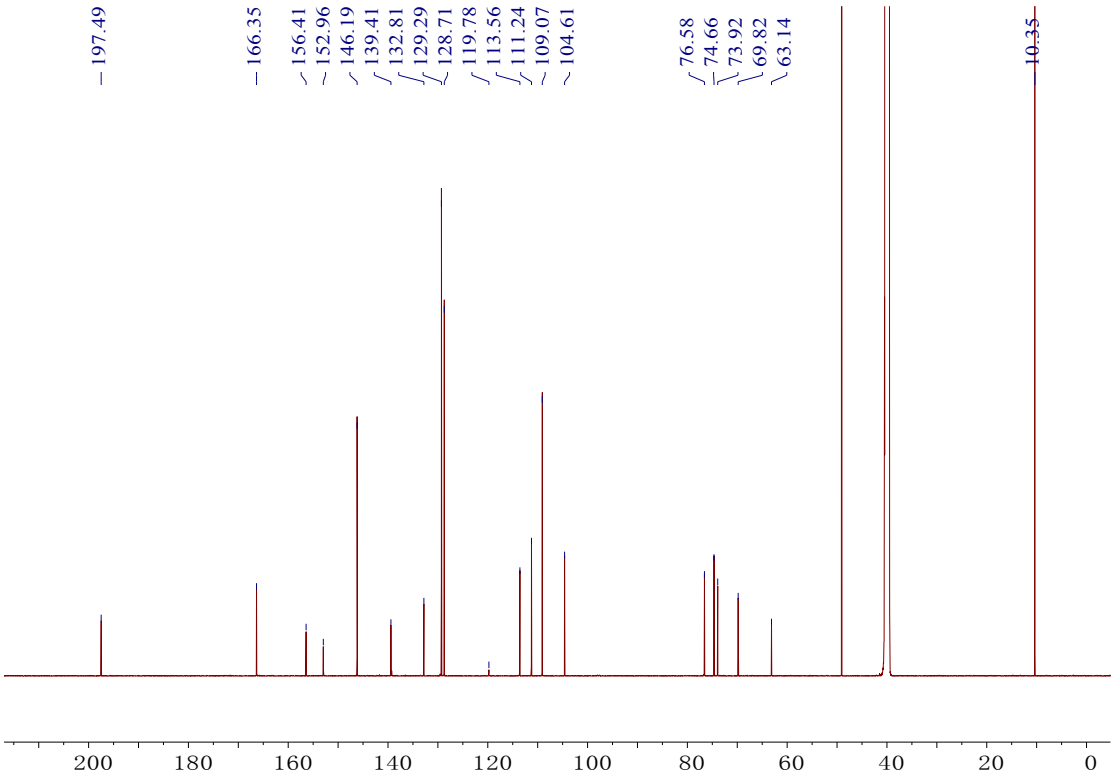


Figure S2. ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of GUB

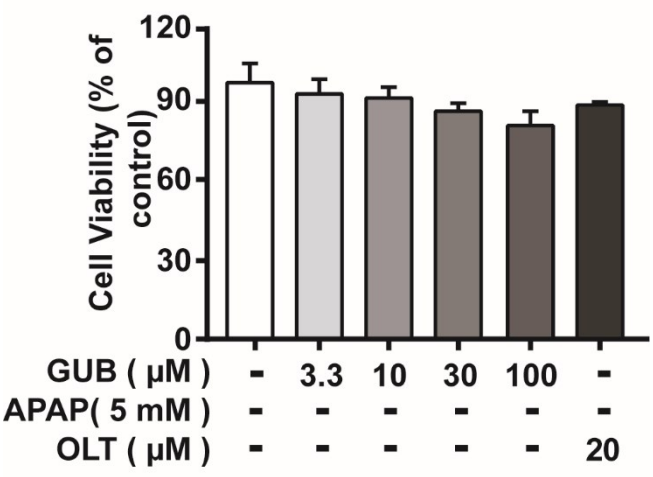


Figure S3. Effect of GUB on cell viability of HepG2 cells. HepG2 cells were seeded at 1×10^4 cells/well in 96-well plates and treated with various concentrations of GUB (3.3, 10, 30, 100 μM) without 5mM APAP stimulation for 24 h. Cell viability was determined by MTT method. Experiment was repeated three times and data were presented as mean ± SE.

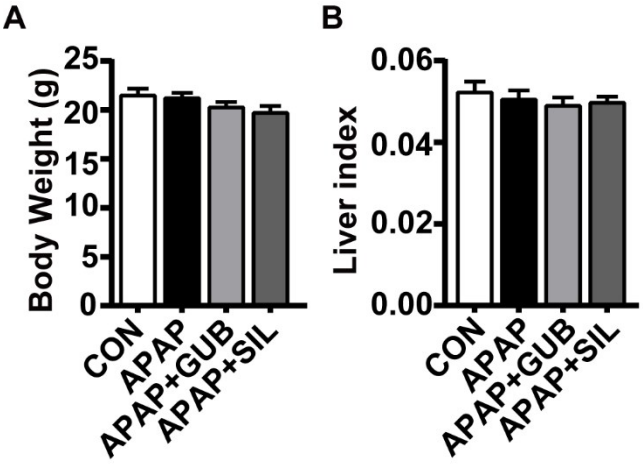


Figure S4. Effects of GUB on (A) body weight and (B) liver index in mice. n = 8-10 per group. Data are shown as mean ± SE.

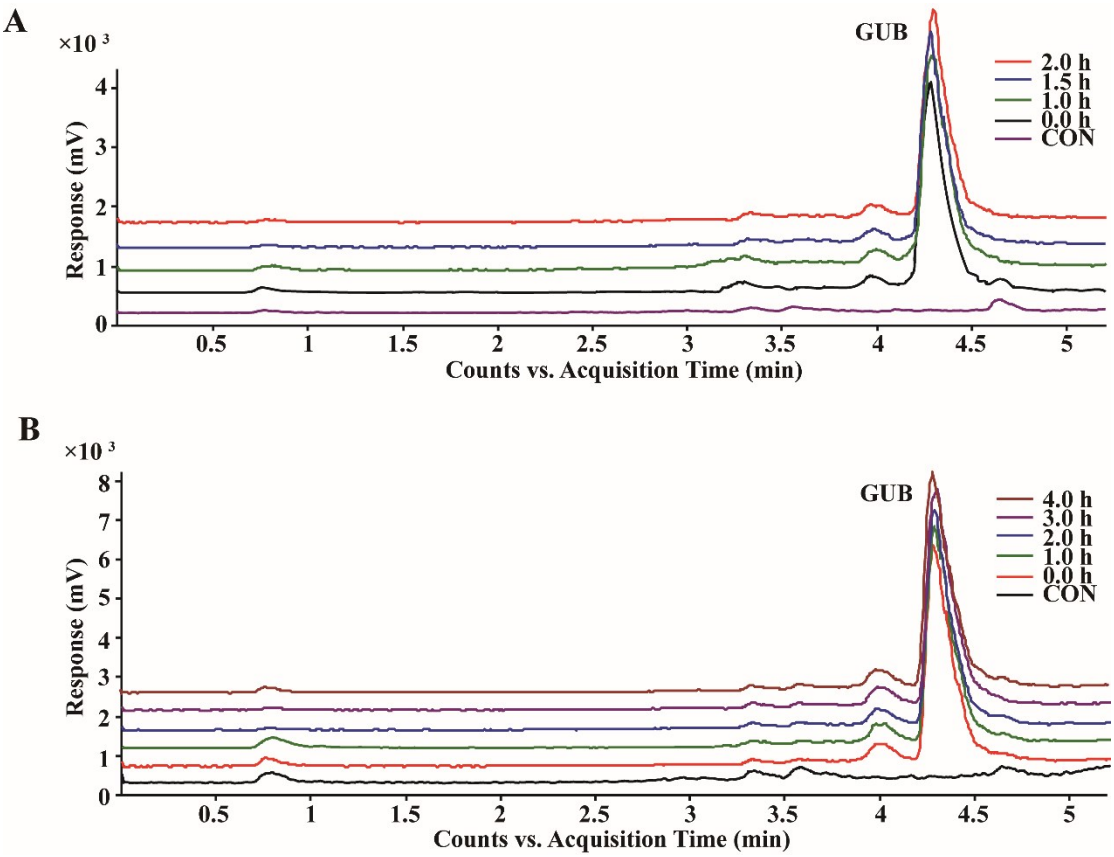


Figure S5. UPLC-MS analysis of GUB after digestion in (A) simulated gastric juice, and (B) simulated intestinal juice.

Table S1 Primer sequences for quantitative real-time PCR.

Genes	Forward	Reverse
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA
GCLC	AACACAGACCCAACCCAGAG	CCGCATCTTCTGGAAATGTT
GPx1	GTGCAATCAGTTCGGACACCA	CACCAGGTCGGACGTACTTG
NQO1	CAGATCCTGGAAGGATGGAA	TCTGGTTGTCAGCTGGAATG
Nrf2	CGAGATATACGCAGGAGAGGTAAGA	GCTCGACAATGTTCTCCAGCTT
SOD1	AACCAGTTGTGTTGTCAGGAC	CCACCATGTTTCTTAGAGTGAGG

Table S2 Analytical parameters of GUB and M2 in quantification experiment

Peak	Rt (min)	$[M-H]^-$	Fragment ions (*m*/*z*)	Calibration curve	R^2
GUB	4.27	571.1	312.9, 257.0, 169.0	Y=0.133620 x + 1452.072083	0.9988
M2	1.37	169.0	125.0, 79.2, 51.1	Y=0.006564x + 48.710514	0.9981

Rt: Retention time, which was shown in chromatogram of UPLC-MS analysis.

The unit for x in Calibration curve is ng/mL.

M2 is represented as gallic acid.

Table S3 Composition of GAM medium

Material	Weight
proteose peptone	10.0 g/L
soybean peptone	3.0 g/L,
yeast extract	5.0 g/L
meat extract	2.2 g/L
digested serum powder	13.5 g/L
liver extract powder	1.2 g/L
glucose	3.0 g/L
KH_2PO_4	2.5 g/L
NaCl	3.0 g/L
soluble starch	5.0 g/L
L-cysteine hydrochloride	0.3 g/L
sodium thioglycollate	0.3 g/L

Dissoved in distilled water